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ARCHIVES OF PATHOLOGY

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THE STERNAL MARROW IN PERNICIOUS ANEMIA

CORRELATION OF THE OBSERVATIONS AT BIOPSY WITH THE BLOOD PICTURE AND THE EFFECTS OF SPECIFIC TREATMENT IN MEGALOBLASTIC ("LIVER-DEFICIENT") HYPERPLASIA

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AND

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Although it may be stated that the blood picture reflects more or less accurately the condition of the blood-forming organs, there is no question but that it leaves much to be desired concerning the underlying pathologic and physiologic conditions. This is particularly true in pernicious anemia. Recent investigations have abundantly shown that this disease represents in all probability a deficiency within the body of certain materials essential for normal cellular development. Lack of these materials results primarily in disturbance of the tongue, the gastro-intestinal tract, the central nervous system and the bone marrow. In the latter tissue the hematopoietic processes become greatly altered, resulting in the characteristic blood picture of the disease: macrocytic, hyperchromic anemia with leukopenia and thrombocytopenia. Since these changes in the blood picture are dependent on the more fundamental changes occurring in the marrow, it was thought desirable to study the marrow at biopsy at various stages of the disease both before and after specific therapy. An investigation of this type might prove helpful in formulating the difference between normal and abnormal, i. e., "liver-deficient," hematopoiesis, in identifying the types of cells which are affected by liver extract and in correlating the reticulocyte response in the blood with the changes occurring simultaneously within the marrow.

Neumann[1] in 1868 first showed that red cells of the blood are formed in the red bone marrow. With the publication of Biermer's[2] article in 1872, pernicious anemia became definitely established as an entity. The first accurate descriptions

From the Medical Service, Department of Pathology and Hematology Laboratory, Beth Israel Hospital.

1. Neumann, E.: Zentralbl. f. d. med. Wissensch. **6**:689, 1868.
2. Biermer, A.: Cor.-Bl. f. schweiz. Aerzte **2**:15, 1872.

of the marrow of patients with this disease were made by Cohnheim [3] in 1876. This writer, in describing the autopsy of a case, observed that the fatty marrow of the long bones was replaced by "a raspberry jelly-like" mass, which on microscopic examination was almost devoid of fat cells and consisted of about equal parts of colorless ("lymphoid") cells and of hemoglobin-containing cells. Cohnheim felt that the alterations in the marrow were the primary process leading to anemia. These observations were abundantly confirmed. Geelmuyden [4] showed that, although the transformation of fatty marrow into "lymphoid" marrow was not a characteristic feature of pernicious anemia since the same process occurred in other types of anemia, it was nevertheless possible to make the distinction by microscopic examination of the nucleated red cells, which were larger in pernicious anemia than in other conditions. Ehrlich [5] in 1892 differentiated sharply between the normal type of red cell formation and that occurring in pernicious anemia. He conceived of two types of erythropoiesis: a normoblastic (or normal) and a megaloblastic (occurring in pernicious anemia). He felt that pernicious anemia was characterized by a fetal type of myeloid activity, in which the nucleated red cells became very large (megaloblasts). This view of the sharp division between the normal and the megaloblastic type of erythropoiesis has become generally accepted. Naegeli [6] has been one of the chief proponents of the view that the megaloblast is peculiar to pernicious anemia (and to fetal red cell formation) and has repeatedly stated that this cell occurs in no other condition. The most thorough and authoritative article on the marrow in pernicious anemia is that published by Ellermann [7] in 1920.

Despite the statement that the marrow in pernicious anemia is megaloblastic, many authors noted dissimilarities in the picture from case to case. Very little attempt was made to correlate these differences with the blood picture, so that the marrow changes present with a changing blood picture could only be inferred. Impetus was given to this phase of the study by the researches of Zadek [8] in 1922. This investigator, by performing biopsies of the tibial marrow in patients in relapse and remission, was able to show striking changes which could be correlated with the findings in the peripheral blood. The intense megaloblastic proliferation of relapse gave way to erythrogenesis of the normal type with onset of remission. When remission was complete, the marrow had assumed its normal fatty appearance, and only an occasional megaloblast could be discovered. Peabody [9] in 1927 extended these observations on tibial marrow and correlated them with the remission induced by liver, the potency of which had only just been discovered.

The introduction of biopsy of the sternal marrow by Seyfarth [10] in 1923 made it a relatively simple matter to study the varying pictures following treatment.

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3. Cohnheim, J.: Virchows Arch. f. path. Anat. **68**:291, 1876.
4. Geelmuyden, H. C.: Virchows Arch. f. path. Anat. **105**:136, 1886.
5. Ehrlich, Paul: Charité-Ann. **13**:300, 1886.
6. Naegeli, O.: Deutsche med. Wchnschr. **26**:287, 1900; Blutkrankheiten und Blutdiagnostik, ed. 4, Berlin, Julius Springer, 1923.
7. Ellermann, V.: Virchows Arch. f. path. Anat. **228**:247, 1920.
8. Zadek, I.: Ztschr. f. klin. Med. **95**:67, 1922.
9. Peabody, F. W.: Am. J. Path. **3**:179, 1927.
10. Seyfarth, Carly: Deutsche med. Wchnschr. **49**:180, 1923.

Many investigators [11] confirmed the original findings of Cohnheim, Ehrlich, Zadek and Peabody. Arinkin [12] in 1929 introduced an even easier method of obtaining marrow material—that of aspiration of the content of the sternal marrow cavity with a hollow needle. By the use of this method, a material which is probably neither marrow nor blood but a variable mixture of both is obtained. Studies comparing the findings in this marrow "juice" with the blood picture have been the subject of many recent articles.[13] The most important of these are by Tempka and Braun,[13a] Nordenson [13d] and Segerdahl.[13f] In addition to these studies on pernicious anemia, studies of the marrow in sprue have been made by Rhoads and Castle,[14] and Miller and Rhoads [15] have made biopsies of the marrow of swine in which this disease had been produced experimentally.

The present report deals with twenty-six biopsies of the sternal marrow of twenty patients, with use of the technic previously described by one of us (Dameshek [16]). Both sections and smears were obtained at various stages of the disease. The findings in the marrow were correlated with the status of the peripheral blood and with the response to treatment. The definite impression was obtained that pernicious anemia is not alone a disease of the red cells but a disease of the white cells and platelets as well. Deficiency in a "liver substance" resulted in profound disturbance in the maturation of these cells with the formation of megaloblasts and of large misshapen granulocytes. The hyperplastic marrow in pernicious anemia was compared with that in other conditions in which deficiency in the "liver substance" was

11. (*a*) Weiner, W., and Kaznelson, P.: Folia haemat. **32**:233, 1926. (*b*) Barta, I.: ibid. **50**:287, 1933. (*c*) Fontana, L.: Arch. per le sc. med. **52**:497, 1928. (*d*) Escudero, P., and Varela, M. E.: Haematologica (II. Recen.) **3**:65, 1932. (*e*) Dameshek, W.: New England J. Med. **205**:1093, 1931; Am. J. M. Sc. **190**:617, 1935. (*f*) Isaacs, R.: Folia haemat. **40**:395, 1930. (*g*) Custer, R. P.: J. Lab. & Clin. Med. **17**:951, 1932. (*h*) Custer, R. P., and Ahlfeldt, F. E.: ibid. **17**:960, 1932. (*i*) Custer, R. P.: Am. J. M. Sc. **186**:617, 1933.

12. Arinkin, M. I.: Folia haemat. **38**:344, 1929.

13. (*a*) Tempka, T., and Braun, B.: Folia haemat. **48**:355, 1932. (*b*) Arjeff, M. J.: ibid. **45**:55, 1931. (*c*) Holmes, W. F., and Broun, G. O.: Proc. Soc. Exper. Biol. & Med. **30**:1306, 1933. (*d*) Nordenson, N. G.: Studies on Bone-Marrow from Sternal Puncture, Stockholm, Generalstabens Litografisha Anstalts Förlag, 1935. (*e*) Krjukoff, A.: Folia haemat. **35**:329, 1928. (*f*) Segerdahl, Elsa: Acta med. Scandinav. supp. 64, 1935, p. 5. (*g*) Rohr, K.: München. med. Wchnschr. **82**:460, 1935. (*h*) Young, R. H., and Osgood, E. E.: Arch. Int. Med. **55**:186, 1935. (*i*) Reich, C.: J. Lab. & Clin. Med. **20**:286, 1934. (*j*) Pokrowsky, E. I.: Folia haemat. **39**:265, 1930. (*k*) Tuschinsky, M. D., and Kotlarenko, B. N.: ibid. **46**:235, 1932. (*l*) Barta, I.: ibid. **50**:287, 1933. (*m*) Kossirsky, J. A.: Arch. f. Schiffs- u. Tropen-Hyg. **27**:496, 1933. (*n*) Amprino, R., and Penati, F.: Minerva med. **2**:463, 1934.

14. Rhoads, C. P., and Castle, W. B.: Am. J. Path. **9**:813, 1933. Krjukoff, A.: Folia haemat. **35**:329, 1928.

15. Miller, D. K., and Rhoads, C. P.: J. Clin. Investigation **14**:153, 1935.

16. Dameshek, W.: Am. J. M. Sc. **190**:618, 1935.

not present. On the basis of the latter observations, it was possible to formulate a simplified classification for normal and abnormal erythropoiesis.

MATERIAL

The patients used in this study all presented typical addisonian pernicious anemia. The diagnosis had been established by physical examination, gastric analysis, studies of the peripheral blood, and of the mean corpuscular volume and the blood bilirubin content. Twenty-six biopsies of the sternal marrow were made in twenty cases of the disease in all stages of remission and relapse. In four cases a second biopsy was made after remission had been established. Biopsies were done in eight instances before institution of therapy, and in eighteen, at some period during therapy. In the latter group, as far as possible, the findings at biopsy were correlated with those in the peripheral blood, with the reticulocyte response, and with the duration of therapy.

METHODS OF STUDY

The method of obtaining sternal marrow for study has been described by one of us (Dameshek) in a previous paper.[16] With the patient under procaine hydrochloride anesthesia, a trephine is introduced into the marrow cavity, and a small plug of bone and marrow is obtained. Bits of bone and curettings from the resultant cavity are used in the preparation of smears. The plug of bone is fixed in Zenker's solution containing glacial acetic acid in the concentration of 5 per cent for decalcification; paraffin sections are made and treated with eosin-methylene blue stain. The smears may be treated with either Giemsa's or Wright's stain. The sections have proved of value in revealing the general topography of the marrow, its cellularity and the presence of islands of primitive cells. The smears, however, have proved invaluable for the careful study of the morphologic characteristics of the individual cells and have accordingly been utilized in making differential counts of the types of cells present. By careful study of both sections and smears one can obtain a far better appreciation of the actual condition of the marrow than is possible by observation of one type of preparation alone.

In the smears, differential counts of at least 500 cells each were made by both observers. The ratio of nucleated red cells to white cells was obtained, together with the percentages of both varieties. The following cell types were differentiated:

(Nucleated Red Cells (Erythroblasts)	White Cells
Megaloblast (A, B, C, of varying grades of maturity)	Histiocyte (hemohistioblast, hemocytoblast, etc.)
Normoblast (A, B, C, of varying grades of maturity)	Myeloblast
	Myelocyte
	Metamyelocyte (normal and abnormal)
	Mature lobulated polymorphonuclear cell
	Eosinophil and basophil
	Lymphocyte

DEFINITION AND DESCRIPTION OF CELL TYPES

Because of the confused state of much of the literature on the subject of erythropoiesis, and because definitions of the terms used

are all too scarce, it has seemed wise to include at this point a description of the various types of nucleated red cells which have been differentiated.

Primitive Red Cell—Erythrogone (Promegaloblast).—This is the most immature cell which can be recognized as belonging to the erythroblastic series. In the marrow of the patient with pernicious anemia, it has usually been termed the promegaloblast. In its most primitive form it resembles in many respects the histiocyte or hemohistioblast (from which it appears to be derived). It differs from the histiocyte by its thick, dense basophilic cytoplasm and well formed nuclear structure. The cell varies in diameter from 19 to 27 microns, the average being 22.7 microns; it is usually round or elliptic in shape with well defined cytoplasmic borders. The cytoplasm is deeply basophilic (deep inky blue with Giemsa's or Wright's stain). The amount of cytoplasm varies with the maturity of the cell; in the most primitive types the cytoplasm is fairly abundant, while in the older cells it may be present as a narrow rim surrounding the nucleus. No granules are present, and no hemoglobin is discernible in the cytoplasm. The nucleus is round and in the more mature type occupies almost the entire cell. The perinuclear membrane is thick and distinct. Surrounding the nucleus, there is often a narrow clear space, usually expanding at one side to form a light *Hof*. The nuclear structure is characteristic, being made up of an arrangement of scroll-like rather pale-staining fine chromatin, resembling a mass of unraveled yarn. It stains light to dark-red violet. From 3 to 5 rather indistinct nucleoli are usually present; these are large, with ill defined borders, and often confluent. They stain somewhat darker than the nuclear structure itself.

In the sections these cells are characterized by the chromophobe character of the nuclei. In these very pale-staining large nuclei the unusually large nucleoli stand out prominently. Mitotic figures are common.

Megaloblast.—The erythrogone by a series of imperceptible transitions becomes the megaloblast. The dividing line between the two types of cells is of necessity at times arbitrary. The megaloblast is definitely more mature, has lost the intense basophilia of cytoplasm characteristic of the erythrogone and is usually without nucleoli. In diameter, it ranges from 13.5 to 25.5 microns, the average diameter being 17.5 microns. It is characteristically oval with a smooth regular border. The cytoplasm varies in amount and in staining with the maturity of the cell. Several shades of maturity may be differentiated. In the most immature type there is relatively little cytoplasm, and this is intensely basophilic and contains only a faint or questionable trace of hemoglobin; in the most mature type the cytoplasm comprises

at least half of the cell area and is orthochromatic (i. e., with its full complement of hemoglobin). Various gradations of color occur between these two extremes. The nucleus is round; it comprises from five to seven tenths of the area of the cell and is composed of a coarse scroll-work, which, once observed, is readily recognized. The more mature megaloblast shows beginning "deterioration" of the scroll-work pattern with the formation of small clumps of material. In the most mature cell the nucleus has shrunk so that it occupies an eccentric position in the cell and comprises only about one fifth of its area. At this stage the cell is distinguishable from the mature normoblast only by its greater size.

The typical megaloblast, when present in fairly large numbers and in association with the erythrogone, may be readily recognized. It cannot be denied, however, that cells resembling the more mature megaloblast are occasionally seen in conditions other than pernicious anemia. It must be admitted that the various criteria for diagnosis even though strictly established cannot always be used to differentiate with certainty between a mature megaloblast and an immature normoblast. This matter will be taken up further in the comment.

Normoblast.—In the so-called normal hyperplasia of erythropoietic tissue seen in severe hypochromic anemia, polycythemia and congenital hemolytic jaundice it is possible to recognize several types of nucleated red cells. The most primitive appears to be identical in all respects with the erythrogone as described. By imperceptible transitions it matures to the normoblast, which is readily recognized. Since these transitions merge so imperceptibly one into the other it has seemed wise not to differentiate the representative cells by name but to use rather the nondescript letters A, B and C. Three main types of normoblastic cells are thus discriminated: A (pro-erythroblast), B (erythroblast) and C (normoblast).

Normoblast A. This cell has been referred to as the pro-erythroblast (Ferrata). It is possible that Naegeli refers to this cell in his description of the so-called macroblast seen in active normoblastic erythropoiesis, although it is more likely that he has reference to the described erythrogone. Normoblast A is seen only in active erythropoiesis, usually of the normoblastic type, although it may be present in considerable numbers in certain cases of pernicious anemia. Two types are present, one round and the other oval or elongated. The round form varies in diameter from 16.5 to 27 microns, the average being 18.9 microns. The cytoplasm is deeply basophilic and without hemoglobin. The nucleus is round and occupies the greater part of the cell. The nuclear chromatin even at this immature state is clearly distinguishable from that of the megaloblast since it is made up of thick lumpy masses of

material without any particular continuity of pattern. The oval type has an intensely blue-staining cytoplasm and often contains from one to three vacuoles and an eccentrically situated relatively small nucleus, in which the clumps of chromatin may be arranged as in the spokes of a wheel. By some authors (e. g., Jordan [17]) this cell is considered to be a plasma cell and thus of the lymphocytic series, and one must admit that the resemblance is frequently striking. The definite hemoglobin coloration in certain of these cells, however, tends to disprove that they are of lymphoid origin.

Normoblast B. In its immature form this cell also resembles closely the plasma cell: It is somewhat elongated with basophilic cytoplasm and without hemoglobin. The cell varies in diameter from 9 to 15 microns, the average being 11.8 microns. The nucleus constitutes only about half of the cell and is composed of thick chromatin blocks, which may be arranged like the spokes of a wheel. As the cell matures the cytoplasm becomes more and more orthochromatic and contains more and more hemoglobin. The nucleus becomes more and more shrunken and the nuclear chromatin more dense. This is the typical so-called erythroblast of the normal bone marrow.

Normoblast C. This is the last stage in the maturation of the nucleated red cell, the cell being only slightly larger than the normal mature red cell and the cytoplasm containing its full quota of hemoglobin. The diameter ranges from 6 to 10.5 microns, the average diameter being 7.7 microns. Nuclear fragments may be present in varying shapes and sizes. The nucleus is small, degenerated and pyknotic.

OBSERVATIONS

Brief protocols of the cases studied and the results obtained in biopsies of the bone marrow are presented in this section. The differential counts of the marrow smears are recorded in table 1.

Untreated Pernicious Anemia.—The following cases illustrate this type of anemia.

CASE 1 (Dec. 4, 1930).—An Irish-born housemaid aged 32 showed the typical features of pernicious anemia with the neurologic signs of severe posterolateral sclerosis. On admission the hemoglobin was 45 per cent; red blood cells, 1,440,000; white blood cells, 4,000; polymorphonuclears, 59.2 per cent (immature 1.6 per cent); lymphocytes, 27.6 per cent; monocytes, 4.8 per cent. Most of the polymorphonuclears showed multilobulated nuclei. The red cells were well filled with hemoglobin and showed striking variation in size and shape. The average red cell diameter was 8.03 microns; the variations were from 3.5 to 13.5 microns. The platelet count was 147,000 (normal about 500,000 per cubic millimeter by the method of Dameshek).[18]

This patient was utilized in testing the therapeutic value of a gastric juice-beef muscle digestion product. A reticulocyte peak of 56.8 per cent was present

17. Jordan, H. E.: Anat. Rec. **42**:91, 1929.
18. Dameshek, W.: Arch. Int. Med. **50**:579, 1932.

on the twelfth day of treatment. The first biopsy of the sternal marrow was made four days before the institution of therapy. The findings in the marrow smears are presented in table 1.

The sections showed definitely hyperplastic marrow, only a few fat cells being present. Megakaryocytes were few. Throughout the section, in islands, were large groups of closely packed cells. These cells were large with relatively narrow rims of basophilic cytoplasm, which were at times irregular in outline. The nuclei took very little stain, showed faint fine reticulum and contained from 3 to 5 prominent nucleoli. Associated closely with these primitive cells (erythrogones) were smaller cells (megaloblasts) in which the nuclear chromatin was more distinct, often in fine particles, and which had less distinctive nucleoli, frequently none. The cytoplasm of these cells, depending on their maturity, contained a varying amount of hemoglobin. Mitotic figures were numerous. Smaller numbers of typical normoblasts with pyknotic nuclei were seen scattered in and about these groups of cells. Increased activity of the leukopoietic cells was also apparent, with predominance in myelocytes and metamyelocytes. The metamyelocytes were often unusually prominent and included many bizarre forms (these are described in the section headed "Analysis of Observations").

Case 2 (Oct. 8, 1929).—The patient, a Jewish tailor aged 62, was given an experimental beef muscle-gastric juice digestion product and showed a reticulocyte peak of 12.4 per cent on the eighth day of treatment.

Case 3 (Jan. 2, 1931).—The patient, an unemployed Jewish woman aged 72, on treatment with a gastric juice-beef muscle digestion product showed only a slight reticulocyte response, to 7.2 per cent; later administration of liver extract by mouth resulted in a reticulocyte peak of 32.6 per cent with rapid rise in the red cell count.

Case 4 (May 21, 1929).—An unemployed Jew aged 64 was treated with liver and liver extract by mouth, and a remission to a normal blood picture resulted. An unusual finding in the marrow on biopsy was large numbers of small round cells (lymphocytes) scattered diffusely throughout the section. Granulopoiesis was about normal.

Case 5 (Aug. 21, 1935).—An unemployed Swedish-American aged 53 was given intramuscular injections of liver extract, and a very slow improvement in the blood picture resulted. The patient's anemia proved very refractory to treatment and at one time relapsed while under treatment (case 26). The marrow on biopsy of smears performed before the institution of therapy showed a mixed megaloblastic and normoblastic type of erythropoiesis (table 1).

Case 6 (June 21, 1935).—An unemployed American aged 62 was treated with liver extract intramuscularly, and a slight reticulocyte response resulted, with gradual remission to a normal blood picture.

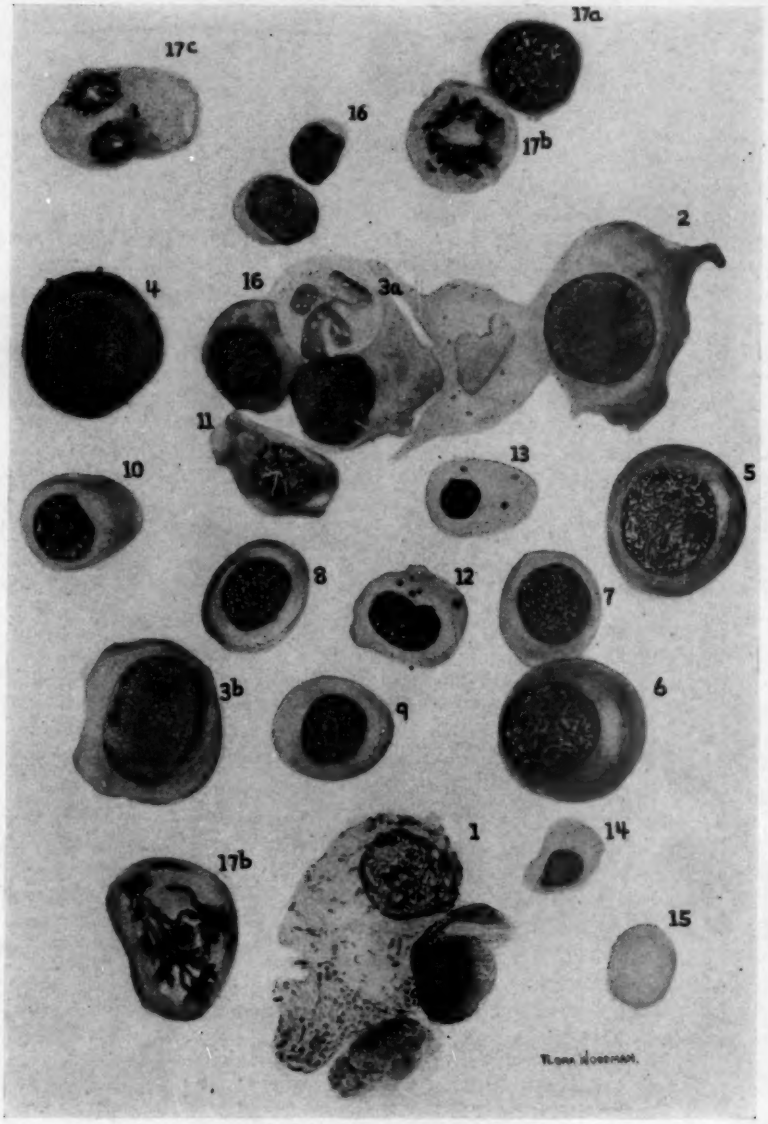
Case 7 (Aug. 19, 1935).—In an American housewife aged 55 parenteral treatment with large daily doses of liver extract resulted in an unsatisfactory reticulocyte response and a very slow rise in the red cell count. Only after several months of persistent parenteral therapy did the count reach normal. The biopsy of the marrow showed only a few megaloblasts, the large majority of the nucleated red cells being normoblastic (table 1).

Case 8 (June 4, 1934).—An Irish-American housewife aged 65 had pernicious anemia that was quite refractory to therapy, the reticulocyte count rising only to 3.3 per cent, where it remained for the remainder of the patient's stay in the hospital. After several months, however, with continued liver therapy the red cell

TABLE 1.—*Differential Counts of the Marrow Smears*

Case	Blood: Red Cell Count	Blood: Hemoglobin,* per Cent	Blood: White Cell Count	Blood: Reticulocytes, per Cent	Blood: Reticulocyte Peak	Blood: Platelet Count	Marrow: Nucleated Red Cells, per Cent: Erythrogones	Megaloblasts	Normoblasts: A	B	C	Leukocytes, per Cent: Myeloblasts	Myelocytes	Neutrophils	Polymorphonuclears	Segmented Forms	Basophils	Eosinophils	Histiocytes	Lymphocytes	R:W Ratio	Time After Therapy	Relation to Reticulocyte Peak
Cases Studied Before Treatment Was Given																							
1 (1st)	1,190,000	45	3,350	0.2	57.0	125,000	31.6	33.3	....	11.1	24.0	2.4	31.9	15.2	18.2	4.3	...	...	...	28.0			
2 (1st)	1,330,000	35	6,500	1.6	12.4	162,000	39.0	49.0	....	....	12.0	5.6	38.2	41.2	....	11.9	...	1.0	2.2	....			
3	1,490,000	50	6,900	2.2	32.6	78,000	35.4	30.4	....	22.1	12.1	0.9	21.5	15.2	21.3	5.5	...	5.0	3.5	27.1			
4 (1st)	1,630,000	37	11,700	0.8	5.5	.....	31.7	46.6	6.4	10.3	5.0	0.3	12.1	10.0	6.2	1.8	0.3	0.9	0.7	67.2			
5	1,740,000	46	4,600	3.7	9.9	.....	17.8	26.3	2.9	28.0	25.0	4.2	20.0	30.2	9.0	3.0	...	1.0	2.7	29.6			
6	1,790,000	49	2,300	...	5.7	209,000	23.2	24.6	2.3	18.6	31.3	1.2	29.8	28.5	19.1	4.7	...	2.8	2.4	12.5			
7	2,690,000	63	3,600	3.9	7.9	143,000	12.8	25.5	18.5	39.7	3.5	2.2	20.2	28.4	7.2	15.6	...	5.8	1.4	19.2			
8	2,780,000	60	7,800	0.9	3.6	353,000	9.2	34.2	10.3	28.3	18.0	2.9	21.2	22.9	6.8	13.2	0.8	10.6	2.0	24.7			
9	3,000,000	72	.....	1.0	5.5	339,000	16.2	36.2	8.8	21.5	17.3	0.9	20.7	30.8	10.6	10.7	...	5.2	1.9	19.4			
10 (1st)	3,270,000	85	.....	...	....	.....	11.6	37.4	5.8	39.4	5.8	1.4	35.6	30.7	4.8	6.4	...	4.3	2.3	14.5			
Cases Studied During Treatment																							
11	1,300,000	20	5,400	0.1	....	74,000	37.0	18.5	15.4	9.7	20.0	2.7	20.1	13.4	19.4	8.3	0.0	2.1	2.1	31.9	1:2.2	36 hr.	No peak
12 (2d)	1,680,000	33	4,000	4.4	....	40,000	37.1	46.8	....	13.3	2.8	0	13.7	35.7	....	12.2	0	2.2	4.9	31.3	1.7:1	48 hr.	5 days before
13	2,130,000	49	3,800	8.3	....	.....	43.1	37.4	1.3	7.8	10.4	1.3	17.8	26.7	13.3	19.2	0	7.6	2.5	12.1	1.8:1	4 days	4 days before
14	1,010,000	40	4,900	9.9	....	.....	19.5	19.3	....	30.6	30.6	1.3	37.8	20.4	0	9.9	0	1.9	1.3	27.4	1.9:1	2 days	3 days before
15	2,240,000	45	8,900	17.0	....	.....	13.5	13.4	....	47.7	25.4	3.6	23.2	26.2	9.0	9.7	0	4.8	3.2	20.3	1:1	7 days	24 hr. after
16	3,550,000	61	7,900	10.1	....	344,000	9.2	0	5.7	56.9	28.2	2.5	32.6	29.6	0	32.5	0	2.8	0	0	1.3:1	9 days	24-48 hr. after
17	1,400,000	42	5,200	5.8	....	.....	17.0	11.1	12.6	35.2	24.1	0	17.6	32.0	2.3	17.6	0	3.3	0.6	26.7	1:1.3	10 days	
18	1,950,000	54	8,700	4.0	....	403,000	2.5	0	19.7	36.8	41.0	2.0	30.0	33.4	5.2	18.2	0	4.2	1.0	6.0	1:3	8 days	3 days after
19	3,660,000	70	9,450	2.2	....	.....	0	0	6.2	46.9	46.9	3.4	22.2	45.4	0	12.6	0	13.3	3.1	0	1:1.4	21 days	8 days after
20 (2d)	3,650,000	69	.....	4.8	....	.....	(Normoblastic marrow with only an occasional megaloblast seen)															22 days	
21 (2d)	3,290,000	70	3,350	0.6	....	.....	1.5	0.2	....	18.9	79.4	1.7	24.0	29.3	7.0	22.5	0	1.3	1.0	13.1	1:2.9	45 days	32 days after
22 (3d)	4,200,000	71	5,000	0.4	....	.....	1.7	0	5.1	29.4	63.8	3.8	25.6	46.5	....	10.3	0	4.7	1.5	7.9	1:1.4	41 days	34 days after
23 (2d)	3,350,000	80	5,400	0.8	....	.....	0.9	1.9	6.7	38.6	51.9	1.3	12.8	22.5	4.0	38.3	0.2	5.5	0.2	15.2	1:6	2 yr.	
24	1,770,000	54	.....	2.1	....	.....	4.2	7.3	18.8	30.7	39.0	1.8	28.0	29.5	15.5	7.9	0	6.0	3.0	8.3	.....	4 days	No response
Cases Studied During Relapse Under Treatment																							
25	2,870,000	65	.....	...	....	.....	21.4	51.7	4.6	10.0	12.3	3.0	18.0	26.7	8.3	15.6	0	4.6	3.7	20.1			
26	2,070,000	75	.....	...	....	.....	16.1	75.5	2.0	3.2	3.2	0.9	18.6	23.8	9.5	13.9	0	5.2	1.7	26.4			

* By Sahli's method (15.5 Gm. is taken as 100 per cent).



EXPLANATION OF FIGURE 1

Photograph of water color painting of selected cells from a smear of sternal marrow from a patient with pernicious anemia in relapse (untreated; case 3); Wright's stain; × 1,000. The successive stages in maturation of the red cells may be followed by the apparent transitions from cell 1 to cell 15 (see text for terminology):

1, histiocyte or hemohistioblast.

2, very early erythrogone.

3a and *3b*, early erythrogones.

4, typical erythrogone (promegaloblast).

5 and *6*, megaloblasts A.

7, *8*, *9* and *10*, megaloblasts B.

11, *12* and *13*, megaloblast C.

14, normoblast C.

15, adult macrocyte.

16, lymphocytes, seen commonly in the marrow of the patient with this disease.

17a, *b* and *c*, megaloblasts in various stages of mitosis.

count had risen to normal figures. The marrow showed, in addition to distinct hypoplasia with apparent increase in the number of fat cells, a scattering of distinct islands of megaloblastic cells.

CASE 9 (Oct. 7, 1935).—An American housewife aged 65 received parenteral treatment with a liver extract and showed a slow but satisfactory remission of the disease. The marrow showed a mixed megaloblastic and normoblastic type of hyperplasia.

CASE 10 (same as case 12).—Biopsy was done because of a relapse three years after the first biopsy.

Treated Pernicious Anemia.—We present several cases of this type of disease.

CASE 11 (July 30, 1934).—An Irish-American housewife aged 37 began to experience malaise, severe nausea and vomiting in the seventh month of pregnancy. Her anemia was exceedingly refractory to therapy, and the reticulocyte count attained a peak of only 9 per cent on the twenty-seventh day of treatment. Subsequently there was complete remission. Biopsy of the marrow thirty-six hours after the institution of therapy (table 1) showed a highly cellular marrow containing islands of very primitive cells (erythrogones) together with groups of actively regenerating leukopoietic cells. The original diagnosis from the sections was acute myelosis (myelogenous leukemia); further study, however, revealed the true erythroblastic nature of the primitive cells.

CASE 12 (Sept. 30, 1931).—An Irish-American housewife aged 53 years, in addition to pernicious anemia, had tertiary syphilis with striking involvement of the bones. She was given daily injections of a dilute extract of liver intramuscularly, with a resultant rise in the reticulocyte curve to 23.6 per cent.

On this admission two biopsies of the marrow were performed, the first one forty-eight hours after the institution of therapy, when the reticulocyte percentage was 2.2, the second on the forty-second day of treatment, when the hemoglobin was 71 per cent and the erythrocyte count 4,200,000. The strikingly dissimilar findings in the marrow smears are recorded in table 1.

CASE 13 (April 6, 1934).—An American saleswoman aged 56 had daily intramuscular injections of a dilute extract of liver and on the eighth day of therapy the marrow showed a reticulocyte peak of 22.4 per cent. Biopsy was done four days after the institution of therapy.

CASE 14 (March 22, 1931).—An Irish-American housewife aged 27 was admitted to the hospital in her fourth pregnancy. In addition, she had active bilateral pulmonary tuberculosis. Gastric analysis showed a small amount of free hydrochloric acid after the injection of histamine. On treatment with liver extract and stomach U. S. P. there was a striking response with a reticulocyte peak of 42 per cent. Biopsy of the marrow, which was done forty-eight hours after the beginning of treatment, when the reticulocyte count was 9.9 per cent, showed extreme hyperplasia of both erythropoietic and leukopoietic centers with the presence of a great many mitotic figures. There appeared to be a mixed megaloblastic and normoblastic type of red cell proliferation.

CASE 15 (Feb. 27, 1931).—The patient was an American-Jewish housewife aged 35. Biopsy of the marrow, performed on the seventh day after the institution of therapy, when the reticulocyte count was 17 per cent, showed only small groups of primitive red cells and megaloblasts, normoblastic cells predominating.

CASE 16 (Sept. 10, 1935).—In an American-Jewish housewife aged 53, biopsy of the marrow, performed approximately nine days after the beginning of treat-

ment and twenty-four hours following a reticulocyte peak of 10 per cent, showed normoblastic hyperplasia; no megaloblasts were seen, although 9.2 per cent erythrogones were present.

Case 17 (Oct. 11, 1935).—An Italian-born housewife aged 65 had had cholecystectomy at another hospital ten months previously. The drainage wound failed to close and continued to drain bile. Biopsy of the marrow, performed ten days after the institution of therapy, showed hyperplastic marrow with small islands of erythrogones and megaloblasts, the latter cells predominating. Normoblastic activity of moderate degree was present.

Case 18 (Dec. 27, 1932).—A Russian-Jewish jobber aged 58 had, in addition to pernicious anemia, chronic bilateral pulmonary tuberculosis and a draining tuberculous fistula in ano. Liver extract therapy resulted in a reticulocyte peak of 22 per cent. Biopsy of the marrow, which was performed eight days after the institution of treatment, when the reticulocytes comprised 12.8 per cent, showed a completely normoblastic type of erythropoiesis.

Case 19 (Aug. 26, 1935).—An unemployed American aged 67 presented the neurologic features of severe posterolateral sclerosis with only mild anemia. The patient was given liver extract parenterally daily in large dosage, with very slow improvement in both the blood picture and the clinical findings. Biopsy of the marrow, which was performed twenty-one days after the institution of therapy, showed complete absence of megaloblasts with a normoblastic type of hematopoiesis.

Case 20 (same as case 4).—The patient improved under treatment, and twenty-two days after the beginning of therapy another biopsy of the marrow was done. At this time the marrow was almost completely normoblastic, only an occasional megaloblast being seen.

Case 21 (same as case 1).—In this case a second biopsy was performed forty-five days after the institution of liver therapy and thirty-two days after a reticulocyte peak of 57 per cent. The marrow smears then showed an essentially normoblastic type of erythropoiesis, only a few megaloblasts being seen.

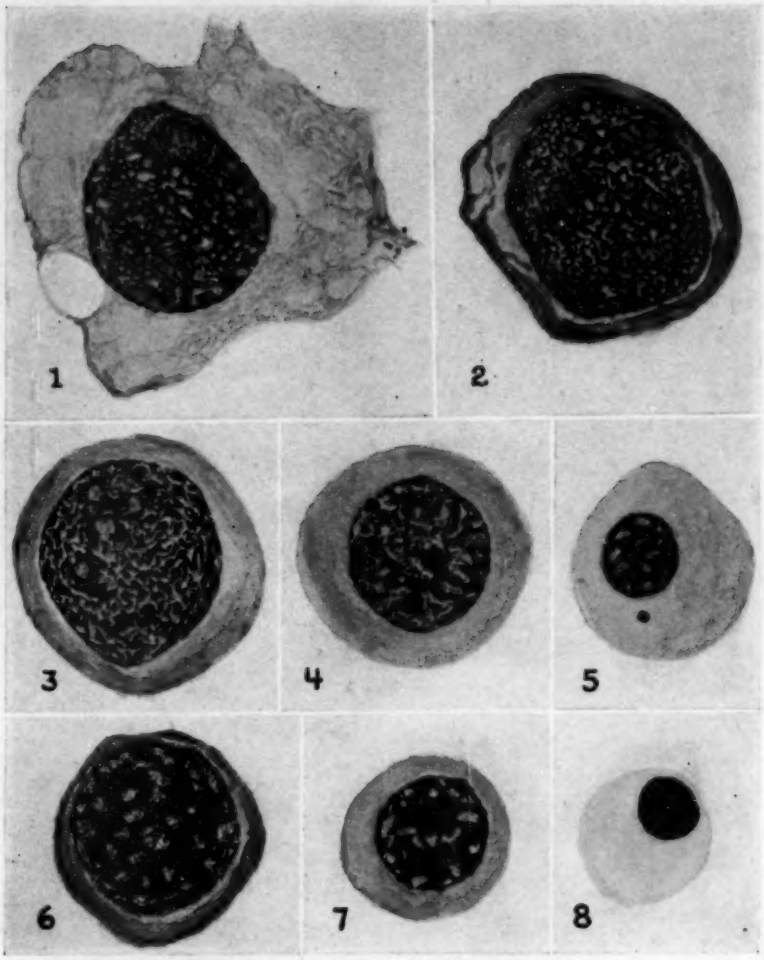
Case 22 (same as case 12).—In this case a second biopsy was made forty-one days after the institution of liver therapy and thirty-four days after a reticulocyte peak of 12.4 per cent. The marrow smears showed an almost completely normoblastic type of erythropoiesis, with only 1.7 per cent of megaloblasts.

Case 23 (same as case 2).—The second biopsy of the marrow was done after the patient had been under treatment for two years and after the recent development of severe combined system disease. The hematologic data were as follows: hemoglobin, 80 per cent; red blood cells, 3,350,000; white cells, 5,800; reticulocytes, 0.8 per cent. The marrow showed normoblastic hyperplasia.

Case 24 (Dec. 5, 1932).—An unemployed American aged 72 presented the findings of combined system disease together with pellagrous lesions of the hands and moderately severe macrocytic anemia. He responded slowly to an experimental gastric extract and to parenteral liver therapy, the greatest reticulocyte percentage being only 2.9 per cent. Biopsy of the marrow, which was performed four days after the institution of therapy, showed only a few megaloblastic cells, the great majority of the cells being normoblasts.

Pernicious Anemia Relapsing While Under Treatment.—In the following two cases relapse during treatment occurred.

Case 25 (May 24, 1934).—An Irish-American janitor aged 37 presented the typical features of pernicious anemia and at first responded very well to parenteral



EXPLANATION OF FIGURE 2

Photograph of water color painting of cells from smears made of the sternal marrow of a patient with pernicious anemia in relapse and of a patient with chronic hypochromic anemia; Wright's stain; × 2,000. The cells are arranged in the order of maturation.

1, early erythrogone or late histiocyte. Note the leukocytoid character of the cytoplasm and the prominent nucleoli.

2, erythrogone. This cell has been called "promegaloblast" when observed in the marrow of a patient with pernicious anemia and "macroblast" when seen in the marrow of patients with other conditions characterized by hyperplasia of the marrow. Such cells appear to be identical in all hyperplastic marrows, whether megaloblastic or normoblastic.

3, megaloblast A, i. e., an early megaloblast. Note the nucleoli.

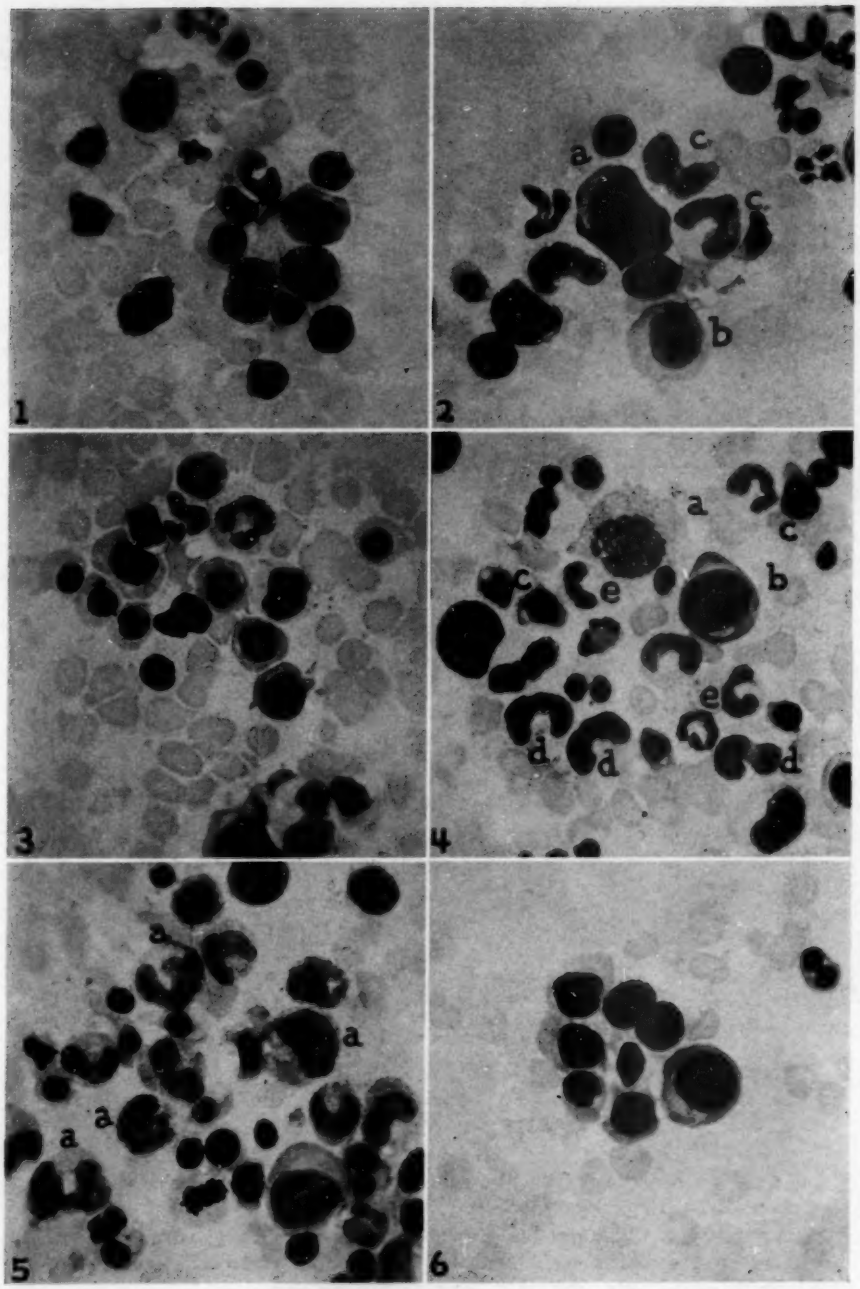
4, megaloblast B, i. e., a megaloblast with a fairly good content of hemoglobin. Note the scroll-work nuclear structure.

5, megaloblast C, i. e., a mature megaloblast.

6, normoblast A, seen in normal hyperplasia. The nuclear chromatin is already somewhat lumpy.

7, normoblast B (erythroblast). The nuclear chromatin shows thick, lumpy structure.

8, normoblast C (normoblast). Observe the pyknotic nucleus.



EXPLANATION OF FIGURE 3

Photomicrographs of cells from smears of the marrow of a patient with pernicious anemia; × 560:

1, group of early megaloblasts with the characteristic scroll-work nuclear pattern.

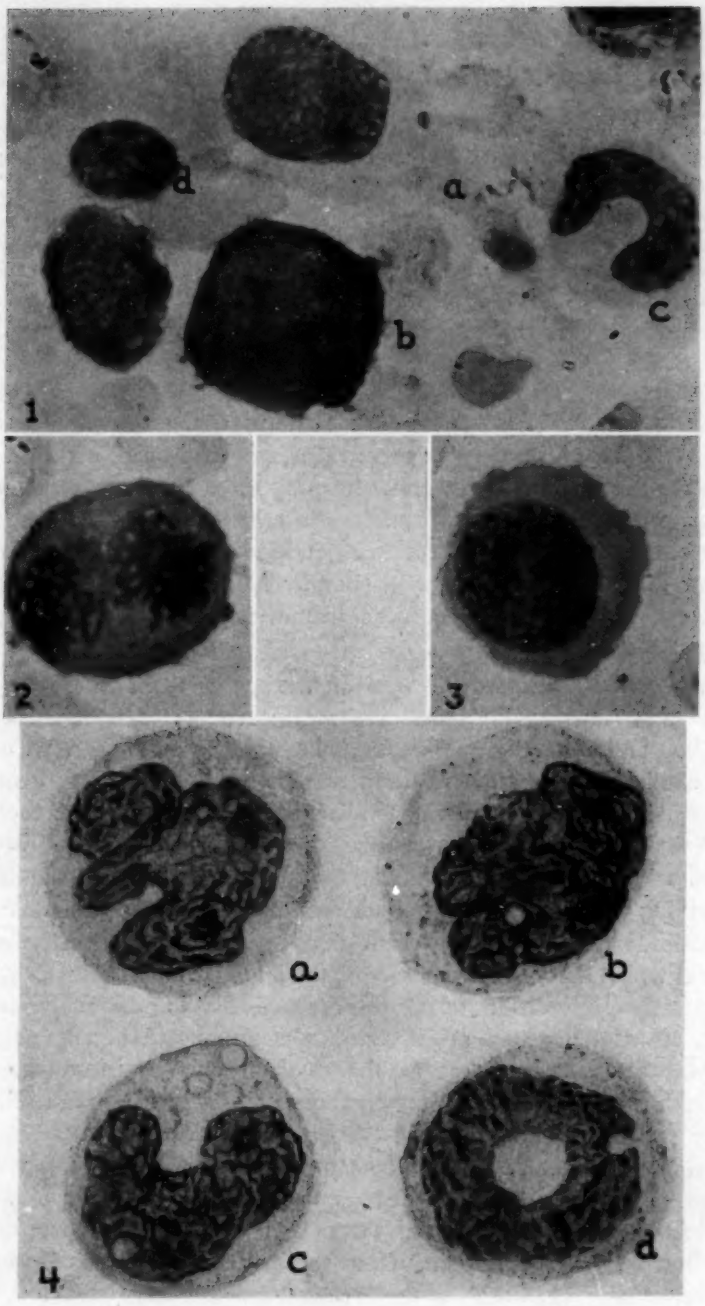
2, (*a*) erythrogone, (*b*) megaloblast B, (*c*) two peculiarly shaped metamyelocytes.

3, a group of megaloblasts in various stages of maturation.

4, (*a*) early erythrogone in early stage of mitosis, (*b*) erythrogone, (*c*) mature megaloblasts, (*d*) pathologic metamyelocytes, (*e*) more normal metamyelocytes.

5, various types of granulocytic cells with several metamyelocytes with bizarre shaped nuclei (*a*).

6, a group of megaloblasts of various shades of maturity.



EXPLANATION OF FIGURE 4

1, 2 and *3,* photomicrographs of cells from smears of the marrow of a patient with pernicious anemia; oil immersion; × 1,000:

1, (*a*) Very large, pale staining early erythrogone (late histiocyte); (*b*) erythrogone (the scroll-work character of the nuclear structure is well brought out; compare with *3;* (*c*) large metamyelocyte; (*d*) lymphocyte.

2, erythogone in mitosis.

3, megaloblast B. Note the scroll-work structure of the nuclear chromatin with the presence of two nucleoli.

4, drawings of various types of large metamyelocytes with bizarre, misshapen nuclei.

treatment with liver extract. He was well for about eighteen months, then, while still under parenteral therapy, he began to relapse. The marrow showed intense megaloblastic hyperplasia (table 1).

CASE 26 (same as case 5).—This patient's anemia proved refractory to all types of therapy, both oral and parenteral. While he was receiving continuous weekly parenteral injections of an extract derived from 100 Gm. of liver, the condition relapsed. A second biopsy of the marrow showed intense megaloblastic hyperplasia (table 1), and the sections showed islands of megaloblastic hyperplasia.

ANALYSIS OF OBSERVATIONS

Erythroblastic Cells.—The most striking changes in the marrow and in the blood in pernicious anemia are concerned with the red cells. As noted in the protocols, the proportion of these cells in the marrow varies considerably from case to case. It is readily seen from analysis of our data that there is definite correlation between the marrow and the blood picture. Generally speaking, the lower the red cell count in the blood the more primitive and hyperplastic is the marrow.

Untreated Pernicious Anemia.—In the ten cases in which the condition was untreated, with erythrocyte counts ranging between 1,100,000 and 2,100,000 per cubic millimeter, the findings were strikingly similar. The sections showed marked hyperplasia, with complete replacement of the marrow by proliferating cells. Many of the cells were obviously very primitive and took the stain poorly. Mitotic figures were numerous. In fact, the appearance of proliferation was so striking at times that one could readily be misled into making a diagnosis of a neoplastic (leukemic) process. In most cases, especially those in which the anemia was severe, the hyperplasia was due primarily to marked proliferation of erythropoietic cells of the megaloblastic series, although myeloid proliferation was also noted in all our cases and was at times as great as the erythroblastic proliferation. The megaloblastic tissue occurred as characteristic islands of large cells, so tightly packed together that the border of each cell at times assumed a polygonal shape. These islands had a bluish appearance as a result of the intense basophilia of the cytoplasm of the erythrogones. The megaloblasts were found about the peripheries of these groups, proliferation apparently occurring from the center outward, as in normal erythropoiesis. The nuclei of the erythrogones and the younger megaloblasts had a chromophobe tendency, taking the stain very poorly, and the former were characterized by the presence of from 3 to 5 large, darkly staining nucleoli.

Examination of the smears showed large numbers of erythrogones (promegaloblasts), and in certain cases, cells even more primitive than these, retaining many of the characteristics of the histiocyte or Ferrata

cell, and yet apparently closely related to the erythrogone in their characteristic nuclear structure and nongranular cytoplasm. Despite the unusual degree of activity in the marrow there was apparently little tendency toward maturation of the red cells, for nucleated red cells of the more mature types were relatively few. This apparent arrest of maturation might account for the great paucity of red cells in the peripheral blood. Furthermore, those megaloblasts showing evidence of maturity in the decreasing size and pyknotic character of the nuclei retained their relatively large size, which undoubtedly accounted for the presence of macrocytes in the peripheral blood.

It should be noted at this point that although various gradations in the maturity of the megaloblasts could be noted, it was almost always possible to differentiate these cells from those of the normoblastic series. Although the characteristic of size is undoubtedly important, we were more impressed by the more fundamental criterion of the configuration of the nuclear chromatin. The delicate scroll-work character of the nuclear chromatin in the erythrogone and early megaloblast was retained almost intact as the cell matured to a stage in which hemoglobin was present. This was in striking contrast to the appearance of normoblastic cells at the same stage of maturity. In the latter, even in the most immature types, the nuclear chromatin was thick and lumpy. These characteristics are brought out in the accompanying illustrations.

At the higher levels of the red cell counts (between 2,500,000 and 3,500,000) the marrow presented less evidence of hyperplasia together with fewer of the most primitive erythroblastic cells (fig. 5). The percentages of erythrogones were considerably diminished, and although megaloblasts of all types were present their number was relatively diminished. There appeared to be at this level a mixed type of erythropoietic activity, in which megaloblastic and normoblastic cells were present together.

It is interesting at this point to compare the observations in the marrow in treated and untreated conditions when the red cell count was about 3,000,000 per cubic millimeter. In two of the cases (7 and 9) biopsy was done before therapy was instituted. In four others (21, 20, 16 and 19) biopsy was carried out after treatment had been under way for some time and the red cell count had reached from 3,130,000 to 3,490,000. The differences are striking. Whereas in the cases in which no treatment had been instituted all the characteristics of the marrow of pernicious anemia in relapse were present, in the cases in which the condition had been treated, *at the same red cell level,* the marrow was essentially indistinguishable from that of the normal person (table 1). Megaloblasts, common in the untreated marrow, were conspicuous in the treated marrow by their absence, and the erythroblastic activity in the latter was almost entirely of the normoblastic type.

Treated Pernicious Anemia.—The observations in the marrow in all the cases in which the condition had been treated are presented in table 1. The data obtained at biopsy have been arranged with reference to the time of the maximum reticulocyte response. This was found to present a closer correlation with the condition of the marrow than the length of time elapsing between institution of therapy and performance of the biopsy. The effect of treatment on the character of the cells in the marrow is brought out in figure 6.

The observations in the marrow in the cases in which treatment had begun varied considerably. In four cases (11, 12, 13 and 14) biopsy was performed at from thirty-six to ninety-six hours after the institution of treatment and before the reticulocyte peak had been reached. In the first three of these cases, the only difference between

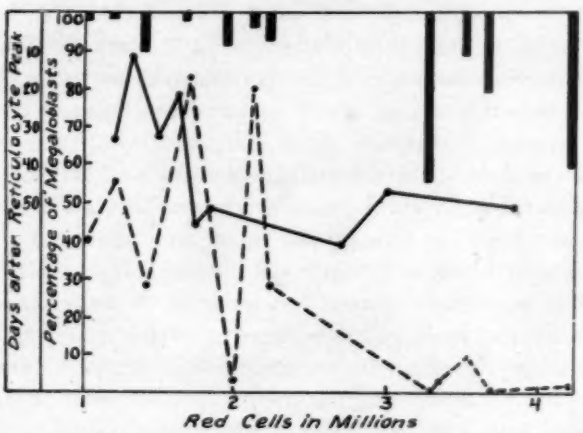


Fig. 5.—The unbroken line represents the percentage of megaloblastic cells in cases in which no therapy had been instituted; the broken line, the percentage of such cells in cases in which therapy was under way. Each dot represents an individual case at the level of the red cell count at which biopsy of the marrow was carried out. The megaloblastic cells comprise both erythrogones (promegaloblasts) and megaloblasts. The vertical columns at the top represent the number of days treatment had been given in the cases in which therapy had been started. Note (*a*) that the largest percentages of megaloblastic cells are present in the cases in which there had been no treatment and in which the red cell counts were lowest; (*b*) that the percentage of megaloblasts remains between 40 and 50 in such cases, in which the red cell count ranges between 1,700,000 and 3,800,000; (*c*) that there is a great scarcity of megaloblastic cells in the cases in which treatment had been begun, in which the red cell count was between 3,000,000 and 4,200,000.

the treated and the untreated marrow at the same blood level was the presence of many more mitotic figures in the marrow under active treatment. In the fourth case (14), with an erythrocyte count of 1,000,000, the marrow showed, only forty-eight hours after the insti-

tution of treatment and two days before the reticulocyte crisis, a definite decrease in cells of the megaloblastic series. In the remaining fourteen cases, observed after the reticulocyte peak had been reached, a rapid decrease, often almost complete, occurred in the numbers of megaloblastic cells during the first few days following the reticulocyte peak. This was accompanied by a concomitant increase in the numbers of normoblastic cells. The normoblastic activity was primarily of the immature variety, but in a few days this activity had subsided to what closely approached the normal status of the marrow with a predominance of mature, i. e., of C, normoblasts. Case 24 must be listed as an exception. The anemia was exceedingly refractory to liver therapy, and the patient's clinical condition and his response to therapy suggested severe vitamin B deficiency rather than classic pernicious anemia.

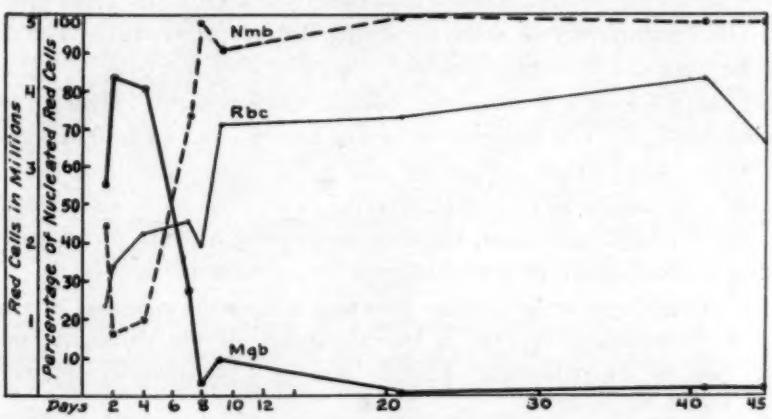


Fig. 6.—Correlation of the megaloblastic and normoblastic cells with the days of therapy. The thick unbroken line represents the megaloblasts; the broken line, the normoblasts. Each dot represents an individual biopsy of marrow. A striking drop in megaloblastic cells with a corresponding sharp rise in normoblastic cells takes place approximately one week after the institution of therapy. This corresponds with about the time of the reticulocyte peak in the blood and before the red cell count (thin unbroken line) has shown decisive increase.

Pernicious Anemia Relapsing While Under Treatment.—Biopsies were done on the marrow of two patients whose condition continued to relapse while under active liver therapy. In both instances there was striking hyperplasia of the marrow with the presence of very large numbers of megaloblastic cells. These were present in larger numbers even than in any of the cases in which biopsy was done before treatment was given. Thus in case 25 we noted 51.7 per cent megaloblastic cells and 21.4 per cent erythrogones, while in case 26 we found 75.5 per

cent megaloblasts and 16.1 per cent erythrogones. The greatest number of megaloblasts in any of the other cases studied was seen in case 2, in which before treatment was instituted and when the red blood cell count was 1,330,000, the megaloblasts numbered 49 and the erythrogones 39 per cent. Thus in the two cases in which relapse occurred while the condition was under active therapy the observations in the marrow were those of unusually severe megaloblastic proliferation.

Granulocytes.—Not only was there hyperplasia of the erythroblastic cells, but leukopoiesis as well appeared to be exceedingly active. Even more significant than this was a distinct qualitative abnormality of granulopoiesis, the most unusual feature being the presence of metamyelocytes of bizarre types. Although Tempka and Braun [13a] described abnormalities in all the cells of the granulocytic series, we observed them chiefly in the metamyelocyte and occasionally in the myelocyte.

The metamyelocytes showed varying degrees of change. The following types of abnormalities were noted:

1. Increase in size over the normal metamyelocyte (from 12 to 15 microns), the cell diameter being frequently from 15 to 30 microns.

2. Increase in both the relative and the absolute size of the nucleus, which was usually large and bizarre in shape, frequently distorted and twisted on itself, and often showing an abortive attempt at hyperlobulation. Occasionally ring or doughnut forms were present.

3. Alterations in the nuclear structure with a disorganized arrangement of chromatin or even a smoothing out of the chromatin mesh and loss of its structure. Certain areas of the nucleus frequently took the stain poorly.

4. Alterations in the cytoplasm, which often appeared to have developed at a slower rate than the nucleus since the basophilia and azurophilic granulation of a much younger cell was frequently retained.

5. The presence of vacuoles, often of large size, in both the nucleus and the cytoplasm.

The myelocytes occasionally showed slight irregularity of the nuclear border, and at times a vacuole was noted in the cytoplasm.

The percentages of these pathologic metamyelocytes varied roughly with the severity of the disease. With a red cell count in the neighborhood of 3,000,000, only a small percentage was present, but with a count of about 1,000,000, these cells comprised about from 10 to 35 per cent of the total number of white cells present. Following therapy these abnormal cells tended to disappear, although their regression was apparently somewhat slower than that of the immature megaloblastic cells.

Lymphocytes.—A striking finding in the marrow of the patient with pernicious anemia is the presence of large numbers of typical lymphocytes having the characteristic size, shape and staining reactions of the mature, or small, lymphocytes of the peripheral blood. Their presence in such large numbers has prompted certain authors to group them with the precursors of the red cells, or erythrogones. They appear, however, to have none of the elements indicating immaturity, either in the cytoplasm or in the nucleus. The cytoplasm of these cells in our preparations was characteristically light blue in its staining, at times showing typical azure granules; the nucleus was round, comprised most of the cell and was composed of thick, heavy blocks of chromatin. In relapse, these cells comprised about 25 per cent of the total number of white cells; with remission after continued therapy they became definitely diminished, in two cases none being seen. Peculiarly enough, with these large numbers of cells, transitions from a more immature type of lymphocyte were not seen. This, together with the lack of clustering of these cells in groups or islands, suggests an infiltration by mature lymphocytes rather than a proliferation of lymphoblastic cells.

Megakaryocytes.—The megakaryocytes in the cases with low red cell counts were either greatly diminished in number or even absent. This finding could be correlated with the low count of platelets in the peripheral blood.

COMMENT

Types of Erythropoiesis; Nomenclature; Erythrogones.—As seen in biopsy and autopsy sections, erythropoietic activity normally takes place in the red marrow of the flat bones and vertebrae. In these situations are found foci of nucleated red cells, which Sabin,[19] Drinker [20] and others have shown are placed intravascularly. These erythropoietic centers are made up chiefly of normoblasts of the most mature type (C), which are only slightly larger than the normal red cells and have small nuclei composed of dense pyknotic material. The cytoplasm usually has its full complement of hemoglobin, although a small percentage of the cells of this type may be polychromatophilic. A few of the cells, especially those situated toward the center of the erythropoietic focus, are slightly larger and apparently less mature. They are characterized by a less pyknotic nucleus, which is larger and composed of dense chromatin masses, often arranged like the spokes of a wheel. In the cytoplasm of these cells (normoblast B; erythroblast) varying amounts of hemoglobin are present. The smears of marrow usually show a greater proportion of the B type than do the sections. In the

19. Sabin, F. R.: Physiol. Rev. **8**:191, 1928.

20. Drinker, C. K.; Drinker, K. R., and Lund, C. C.: Am. J. Physiol. **62**:1, 1922.

processes of preparation of the sections, it is likely that the nuclear chromatin shrinks to a far greater degree than is indicated from examination of the smears.[21]

In the normal marrow about 70 to 90 per cent of the cells are mature, and from 10 to 30 per cent are somewhat immature normoblastic cells. Cells excessively immature, with intensely blue cytoplasm or with nucleoli, are only occasionally seen. In conditions associated with increased formation of red cells (except pernicious anemia), as hemorrhage, severe primary (idiopathic) hypochromic anemia,[21a] congenital hemolytic jaundice and polycythemia, normoblastic activity appears to be greatly intensified. There is a shift to cells which are definitely immature, the earliest types of which contain no hemoglobin in their cytoplasm and may contain nucleoli. The percentage of mature cells (C) becomes greatly reduced. Definitely more immature than the B type of normoblast described is a large cell about the size of a megaloblast or larger (from 16 to 27 microns) with a cytoplasm staining deep blue and a round nucleus, which occupies about five to seven tenths of the area of the cell and shows lumpy chromatin. An occasional cell of this type may superficially resemble the megaloblast, but careful examination of the nuclear chromatin should correct this impression. The megaloblastic nucleus even in its most mature form preserves its scroll-work appearance, which, once appreciated, can readily be differentiated from the gross lumpy appearance of even the most immature normoblastic cell.

It is certain, however, that in conditions of most intense hyperplasia, some cells are present which are in every respect identical with some of the cells seen in pernicious anemia. These cells are chiefly characterized by the intense basophilia of the cytoplasm, by the small amount of the cytoplasm and by the large nucleus composed of a fine chromatin mesh (scroll-work) always containing nucleoli. Naegeli[6] refers to these cells as "macroblasts"; Ferrata and Rinaldi[22] have called them

21. A number of observers have commented on the fact that smears are infinitely more valuable for studying the fine cellular morphology of the bone marrow than are sections. Thus, N. A. Michels (Folia haemat. **45**:75, 1931) stated that "Pappenheim, Ferrata, Schridde, Naegeli, Klein, and others criticized Maximow's work to the effect that his persistent unilateral slant on the all sufficiency of sectioned material prevented him often from properly evaluating the importance of nuclear morphologic changes as specific expressions of cell ancestry and maturation. Recently Downey in a study of the myeloblast gives a similar view. Most severe is perhaps Naegeli, who is of the conviction that in sections it is absolutely impossible to distinguish basophilic, deep polychromatophilic erythroblasts from myeloblasts or lymphocytes . . ."

21a. Dameshek, W.: J. A. M. A. **100**:540, 1933.

22. Ferrata, A., and Rinaldi, de Negreiros: Virchows Arch. f. path. Anat. **215**:77, 1914.

"proerythroblasts" and Helly [23] used the noncommittal term "erythrogone."

In the marrow of pernicious anemia, Ferrata and Rinaldi [22] and following them many others referred to cells identical with these as "promegaloblasts." On the basis of a careful study of these cells, particularly of those from our smear preparations, we have been led to the conclusion that the most primitive cell which can be recognized as a nucleated red cell and not a leukocyte is identical in both normoblastic hyperplasia and the hyperplasia associated with pernicious anemia. We have preferred to use for this type of cell Helly's original term, "erythrogone." Helly [23] pointed out the identical nature of these cells in all states of what he termed "anemic degeneration," which he found in the marrow of persons suffering from hemorrhage, lead poisoning and pernicious anemia. The almost leukocytic character of the cytoplasm of these cells led Cohnheim [3] and others to refer to them as "lymphoid cells." Ellermann,[7] in his very thorough article, analyzes the various views which are held regarding the nature of these cells. Wolff,[24] Naegeli [6] and, following the latter, Schridde [25] and Nicol [26] felt that these cells were myeloblasts which became differentiated either to granular cells or to megaloblasts. Pappenheim,[27] Klein [28] and possibly Ferrata and Rinaldi [22] believed that these cells were capable of development in any direction, i. e., to red cells, granulocytes or lymphocytes. Güse [29] in a recent article attempted to revive this view. Pappenheim called these cells "lymphocytes," differentiating them, however, from the lymphoblasts found in lymphoid tissue. Helly,[23] Ellermann,[7] Barta [11b] and Krjukoff [13e] all concluded that these cells were erythrogones, i. e., primitive red cells, capable of developing only into red cells. Ellermann in combating Naegeli's views regarding the myeloblastic character of these cells pointed out that (1) they appeared in groups surrounded by megaloblasts; (2) transitions between them and megaloblasts could readily be seen; (3) in the liver and spleen they assumed an intravascular position characteristic of red cells; (4) unlike granulocytic cells, they were often arranged in syncytial layers, and (5) the angle of the mitotic spindle in the erythrogone was similar to that seen in the typical megaloblast, and the same coarse and sharply defined spindle threads were present. To these differentiations may be added

23. Helly, K.: Beitr. z. path. Anat. u. z. allg. Path. **49**:15, 1910.

24. Wolff, E. K., in Hirschfeld, H., and Hittmair, A.: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwartzenberg, 1933, vol. 1, pt. 2, p. 1089.

25. Schridde, A.: Folia haemat. (supp. 2) **4**:151, 1907.

26. Nicol, K.: Deutsches Arch. f. klin. Med. **11**:417, 1913.

27. Pappenheim, A.: Virchows Arch. f. path. Anat. **157**:19, 1899.

28. Klein, E.: Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl., Wien, 1871, vol. 63.

29. Güse, T.: Deutsches Arch. f. klin. Med. **177**:316, 1935.

the further data derived from our studies that in smear preparations of the marrow the cytoplasm of the erythrogone is more abundant and stains a more intense inky blue than that of the myeloblast. Furthermore, the scroll-work nucleus is quite different from that of the myeloblast, which contains an exceedingly fine network, in which the threads are not readily discernible. In the latter cell, furthermore, oxidase granules are usually present at least to some extent even in the most primitive forms.

The erythrogones are found in all conditions in which there is intense erythropoietic activity. In their manner of differentiating to more mature types, however, these cells differ strikingly. In conditions of simple hyperplasia the next most mature cell type contains a definitely lumpy nuclear chromatin (normoblast A). In the abnormal hyperplasia of pernicious anemia all successive cell types contain the original scroll-work type of nuclear chromatin seen in the erythrogone, although the cytoplasm gradually becomes modified. These cells are ordinarily larger than comparable cells of the normoblastic series. Together with the criterion of size, which we feel is not so important as that of nuclear morphology, these cells are recognized as megaloblasts of varying degrees of maturity. We have used the noncommittal terms "A," "B" and "C" for describing successive changes in maturity of these cells. The most mature type has a pyknotic nucleus and except for the matter of larger size and somewhat large nucleus cannot be distinguished from normoblast C. Megaloblast B has a fairly high content of hemoglobin in its cytoplasm and a nucleus which occupies about half the cell. Megaloblast A has either a faint trace of hemoglobin or none at all and a larger nucleus. In the more mature megaloblast, although the scroll-work character of the nucleus is preserved, the chromatin tends to be more thickened and much less delicate than that in the immature types. It is interesting to observe the preservation of the scroll-work character of the nuclear chromatin even in the more mature types.

The normal marrow gives rise to mature nonnucleated red cells, which average 7.5 microns in diameter. In conditions of simple hyperplasia, the mature red cells which are formed are never larger than normal, although they are frequently smaller. Microcytosis and hypochromia are the common derivatives of a simple hyperplastic marrow; these are probably indexes of excessive cellular production in the absence of a sufficient excess of hemoglobin for the enormous number of cells being produced. In pernicious anemia, on the other hand, the end-result of the definitely abnormal megaloblastic hyperplasia is a large red cell (macrocyte) which is well filled with hemoglobin. The successive stages in the maturation of red cells in normal marrow, simple hyperplastic marrow and the marrow that shows pernicious anemia are depicted in table 2.

From this table it may be seen that for practical purposes erythropoiesis may be classified either as normoblastic or megaloblastic. The erythrogone, the most primitive nucleated red cell, has two types of potentialities: a normal (normoblastic) and a "liver deficient" (megaloblastic) development. We feel that this classification, although it is based purely on morphologic observations, embraces certain physiologic principles as well. It has, furthermore, the advantage of relative simplicity and avoids many controversial terms such as "promegaloblast," "macroblast," "pro-erythroblast" and "erythroblast." It is probably better, we feel, to reserve the term "erythroblast" as a general one

TABLE 2.—*Authors' Conception of the Various Types of Erythropoiesis: (a) Normal, (b) Normal (Normoblastic) Hyperplasia, (c) "Liver-Deficient" (Megaloblastic) Hyperplasia*

	Normal (Normoblastic)	Normal (Normoblastic) Hyperplasia	"Liver-Deficient" (Megaloblastic) Hyperplasia
		Chronic hemorrhage Hypochromic anemia Polycythemia Congenital hemolytic icterus	Pernicious anemia and allied states
Marrow		Erythrogone	Erythrogone (Promegaloblast)
		Normoblast A (Pro-erythroblast) (Macroblast)	Megaloblast A
	Normoblast B (Erythroblast)	Normoblast B	Megaloblast B
	Normoblast C (Normoblast)	Normoblast C	Megaloblast C
Blood	Normocyte (7.5 microns)	Normocyte Microcyte (6 microns or less)	Macrocyte (9 microns or over)

embracing all types of precursors of the red cells. The erythropoietic cells as we have classified them are as follows:

Erythroblast. A generic term embracing all red cell formers

Erythrogone (promegaloblast; macroblast). The most primitive nucleated red cell, seen in conditions of hyperplasia of the marmarrow, both normoblastic and megaloblastic

Megaloblast A. Immature megaloblast

Megaloblast B. Moderately mature megaloblast

Megaloblast C. Mature megaloblast with a practically pyknotic nucleus

Normoblast A (pro-erythroblast). A cell seen only in normoblastic hyperplasia

Normoblast B (erythroblast). A cell seen normally as constituting about 10 to 30 per cent of erythropoietic cells

Normoblast C (normoblast). A cell with a pyknotic nucleus

Nature of the Megaloblastic Proliferation in Pernicious Anemia; Specificity of the Megaloblast.—Erythropoiesis in all conditions except pernicious anemia is always of the normal (normoblastic) variety. Pernicious anemia may be considered as a deficiency disease in which the body fails to receive a normal amount of what is conveniently designated as a "liver substance." Castle and his co-workers [30] showed that the stomach is all-important in the normal digestion of protein, another substance being formed which on absorption by the intestine is active in the stimulation of normal hematopoiesis. Castle's experiments led him to incriminate the stomach ("intrinsic factor") as the chief defective link in normal erythropoiesis, but he also emphasized the importance of an adequate diet ("extrinsic factor") and of a normally absorptive intestinal tract.[30b] Recent investigations have emphasized the importance of the dietary factor and have illustrated the development of macrocytic, hyperchromic anemia under conditions of diverse etiology. One of us (Dameshek [31]) has recently summarized the etiologic factors that are important in the development of pernicious anemia. In brief, these may be dietary, gastric, intestinal, possibly hepatic, and in certain cases perhaps associated with pregnancy, syphilis or tuberculosis. It is probable that the presence of numerous etiologic factors is necessary before actual deficiency in the "liver substance" results.

The changes in the marrow in pernicious anemia have often been likened to the type of erythropoiesis seen in the embryonic liver. In our observations of a few fetal livers we were struck by the pernicious anemia-like character of the erythroblastic cells. The erythrogones were prominent and appeared to mature not only to megaloblastic but to normoblastic cells. Wintrobe and Schumacker [32] in a recent paper presented certain observations which may have some bearing on these points. In a study of the blood obtained from various types of fetuses, both human and animal, they found macrocytic anemia, which diminished with increasing maturity of the fetus. On the basis of numerous correspondences of fetal blood with that of persons with pernicious anemia, they postulated that the fetus might be suffering from a "liver deficiency" which gradually lessened with increasing maturity. According to these observations, it seems that pernicious anemia does not represent a reversion to a fetal type of cell growth but that in both the patient with pernicious anemia and the normal fetus the megaloblastic type of erythropoiesis is dependent on "liver deficiency."

30. (*a*) Castle, W. B.: Am. J. M. Sc. **178**:748, 1929. (*b*) Castle, W. B.; Rhoads, C. P.; Lawson, H. A., and Payne, C. C.: Arch. Int. Med. **56**:627, 1935.

31. Dameshek, W.: New England J. Med. **213**:805, 1935.

32. Wintrobe, M. M., and Schumacker, H. B., Jr.: J. Clin. Investigation **14**: 837, 1935.

In the presence of this deficiency a striking change occurs in the marrow, a change which is, however, not irreversible and which is almost immediately influenced by liver extracts. This change is characterized by the presence of large numbers of megaloblasts and erythrogones. Great hyperplasia of these cells in the marrow at a time when the mature red cells in the peripheral blood are gradually diminishing in number suggests that an arrest of maturation is present. The presence of megaloblasts, cells which are foreign to normal hyperplasia, suggests that together with the arrest of maturation there is an altered form of erythropoiesis.

When the anemia is milder there is a less fundamental alteration in the erythropoiesis, so that side by side with megaloblasts there are numerous normoblasts. Krjukoff [13e] was able to study a patient with relapse of sprue in a very early stage who had macrocytic anemia (red cells, 4,500,000); he found that even at this early stage of relapse megaloblastic cells were present among the predominant normoblastic cells. Ellermann [7] stated that normoblastic as well as megaloblastic cells may be present simultaneously. Barta [11b] pointed out that megaloblastic and normoblastic activity go on side by side; which is greater depends on the severity of the anemia. It seems that in pernicious anemia normoblastic and megaloblastic activity are related in a reciprocal manner; the greater the degree of megaloblastic activity the fewer normoblastic cells are seen and vice versa. A complete lack of normoblastic activity must indeed be very rare.

With continued "liver deficiency," it seems that larger areas of the marrow become involved; there is intense hyperplasia with involvement of normally inactive areas, as in the yellow marrow of the femurs. Despite this great cellular growth in the marrow, the number of cells in the peripheral blood constantly diminishes until finally severe anemia is present. Marrow activity may be said to be "frozen," and what growth takes place is definitely abnormal in type with the final stage of maturation a hypervolemic macrocyte.

Other Changes in the Sternal Marrow.—The two outstanding end-results of "liver deficiency" as it affects the marrow appear to be arrest of maturation and maldevelopment. Not only are the red cells affected, but, as already noted, the leukocytes show marked changes. These changes have been commented on by Ellermann, Tempka and Braun, Nordenson and Segardahl. The frequent presence of myeloid hyperplasia together with leukopenia in the peripheral blood is again indicative of an arrest of maturation. The huge bizarre forms of metamyelocytes suggest monstrosities in cellular development. It is likely that the many-lobed mature polymorphonuclear cells of the peripheral blood are indicative of cellular maldevelopment from the peculiar meta-

myelocytes of the marrow rather than of excessive maturity as is ordinarily stated. The apparent multiplicity of nuclear lobes may be due to the twisting or breaking up of the extremely large nucleus of the metamyelocyte.

The changes in the white cells together with the diminution in the number of megakaryocytes (and blood platelets) suggest strongly that pernicious anemia is a disease not alone of the red cells of the marrow but of the white cells and megakaryocytes as well. This may be inferred from examination of the peripheral blood. Prior to treatment, the total leukocyte count is reduced because of an absolute reduction in polymorphonuclear cells; these are frequently multilobulated and occasionally very large. The platelets are usually reduced to a half or a quarter of their normal number and at times even below the so-called critical level, so that petechial hemorrhages may take place. Following therapy, not only does the number of red cells increase, but the leukocyte count rises sharply with the presence of large numbers of immature polymorphonuclears. The platelet count may begin to rise even before the first rise in the reticulocyte count; at any rate, almost invariably the rise in platelets keeps pace with the rise in reticulocytes until finally there is a normal or even an increased platelet count.[33]

The foregoing observations strongly suggest that in the state conveniently designated as "liver deficiency" an abnormal type of hematopoiesis takes place, the most striking feature of which is megaloblastosis in the marrow and macrocytosis in the circulating blood. The megaloblast may thus be said to be the specific cell of "liver deficiency." Theoretically it should appear in all conditions in which for some reason there is an insufficient amount of the "liver substance" appearing in the marrow. Actually megaloblastosis and macrocytosis occur in certain cases of dietary deficiency, gastric abnormality, severe diarrhea and combinations of these factors. Whether the macrocytic anemia of hepatic disease is associated with a megaloblastic marrow remains to be seen.

The finding of megaloblasts by various observers in conditions other than the so-called liver deficient state suggests either that the various criteria for cellular diagnosis have not been observed or that an unrecognized deficiency of the "liver substance" is present. We are agreed with Naegeli in his emphatic statements that the megaloblast is specific for pernicious anemia (and allied states). Michels [34] in his review of

33. This view of the involvement of the whole marrow in pernicious anemia has also been pointed out in a very recent communication by Jones (Proc. Soc. Exper. Biol. & Med. **34**:694, 1936), who concludes that the lack of antipernicious anemia principle produces panmyelopathy in that three distinct cell lines are affected.

34. Michels, N. A.: Folia haemat. **45**:75, 1931.

the literature on erythropoiesis made much the same point and stated, "Accordingly, adult bone marrow contains neither megaloblasts nor megalocytes. Structures simulating them are to be interpreted as macronormoblasts, normocytes."

Effect of Liver Therapy on Megaloblastic Marrow.—As noted by Peabody [9] shortly after the advent of liver therapy and confirmed by other observers, a striking change in the character of the marrow occurs following the administration of the specific "liver substance." This change takes place at least within a few days, as observed in our own cases, and may even occur within twenty-four hours, as suggested by Segerdahl [13f] in her studies with marrow punctures. The most thorough studies of the effects of therapy have been made by Nordenson,[13d] who was able to make successive punctures of the sternum in a large series of cases. His observations are in general similar to our own. He found that the changes in the marrow had reached their maximal intensity on the third or fourth day of treatment, a few days before the reticulocyte peak in the blood. He stated that both during relapse and in early remission the marrow is hyperplastic.

The marrow, which first is composed in great part of exceedingly primitive nucleated red cells (erythrogones) and of megaloblasts, rapidly gives way to an active marrow in which the cells are for the most part normoblastic. It is uncertain from our studies whether the erythrogones are led by the action of liver to develop normally into normoblastic cells, whether megaloblasts are directly modified to normoblasts, or whether both processes go on hand in hand. It seems to us that the latter possibility is most likely, since the percentages of both erythrogones and megaloblasts rapidly diminish. The effect of the liver therapy seems to be on those cells which are suffering either from an arrest in development or from faulty development, with the result that the "frozen" hyperplasia in the marrow is broken up, and maturation takes place rapidly with resultant reticulocytosis in the blood, the final step being the production of extremely large numbers of red cells in a short space of time. The red cell count in the peripheral blood may increase from 1,000,000 to 5,000,000 per cubic millimeter in from four to eight weeks. If it is assumed that the total blood volume is about 5,000 cc., the total number of cells produced is almost beyond comprehension. That this is possible is undoubtedly due to the already hyperplastic condition of the marrow over an abnormally large area. The chemical nature of the response to liver extract in the "liver-deficient" megaloblasts awaits future study.

Along with this rapid change in the erythroblastic elements of the marrow occur striking changes in the character of the leukocytic picture:

a disappearance of the misshapen giant polymorphonuclear cells and of the lymphocytes which are so commonly seen. Again, this serves to bring out that pernicious anemia is a "liver deficiency" disorder in which the entire marrow is affected and which is to be relieved only when specific therapy is applied.

SUMMARY

Biopsies of the sternal marrow were made in twenty cases of pernicious anemia at various stages both before and after treatment with antianemic substances. Both sections and smears made directly from the marrow were studied. For exact cellular differentiation and for differential counts, the smears were found to be much more satisfactory than the sections.

The changes in the marrow were correlated with the status of the peripheral blood. In the cases in which treatment had been given the findings varied according to the time of the biopsy in relation to the reticulocyte peak. Before the reticulocyte peak the marrow showed evidence of marked activity with many mitotic figures. Within a few days after the reticulocyte peak there was almost complete disappearance of megaloblastic cells, accompanied by a concomitant increase in the numbers of cells of the normoblastic series. In two cases in which the condition relapsed while under active parenteral treatment with liver, intense megaloblastic hyperplasia was present.

An associated hyperplasia of the granulocytic cells was observed together with the presence of many bizarre forms of metamyelocytes and large numbers of typical lymphocytes.

On the basis of the aforementioned and correlated observations, it may be stated that there are two types of erythroblastic hyperplasia; (1) the normoblastic (occurring in iron-deficient states, polycythemia, congenital hemolytic icterus, etc.) and (2) the megaloblastic (occurring in "liver deficient" states, or pernicious anemia). The most primitive red cell present in both these conditions is the erythrogone.

In the state of "liver deficiency" (pernicious anemia), erythropoiesis becomes arrested at the erythrogone level, and whatever maturation takes place is in the direction of the abnormal megaloblast, the last stage of which is the macrocyte. Thus, both arrest of maturation and maldevelopment are present. This is also exemplified in the condition of the granulocytic cells, which often show monstrosities of cellular development, the end-results of which are probably the many-lobed polymorphonuclear cells of the peripheral blood.

The changes in (1) the erythroblastic cells, (2) the granulocytes and (3) the megakaryocytes suggest that pernicious anemia is a disease affecting the entire marrow. Other studies have shown that this is

dependent on a deficiency in what is conveniently called a "liver substance." The striking and almost incredibly rapid effects of liver extract on the marrow are seen in (1) the disappearance of erythrogones and megaloblasts and their conversion to normoblasts, (2) the disappearance of abnormally shaped granulocytes, (3) the disappearance of lymphocytes and (4) the increase in the number of megakaryocytes. Liver extract appears to supply a material which is necessary for the normal development of all the cells in the marrow. In the absence of this substance arrest of maturation and maldevelopment take place. This is reflected in the peripheral blood as anemia of the macrocytic type, leukopenia with the presence of many-lobed (pernicious anemia) neutrophils and thrombocytopenia.

CEREBRAL LESIONS IN HYPOGLYCEMIA

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MINNEAPOLIS

Within recent years it has been recognized that many patients presenting varied and bizarre neurologic symptoms are suffering from hypoglycemia. In most instances in which the patients have presented themselves to neurologists, the latter, accustomed to thinking primarily in terms of diseases of the nervous system, have classified the conditions observed as idiopathic epilepsy, cerebral neoplasm, encephalitis, alcoholic intoxication, neurasthenia, hysteria, etc. (Womack, Gnagi and Graham;[1] McClenahan and Norris;[2] Carr and others;[3] Nielson and Eggleston;[4] Cammidge[5]).

The widespread and often persistent findings in the patient with hypoglycemia point very strongly to some definite change within the central nervous system. The nature of this alteration is at present only slightly understood. During the past few years sporadic reports have appeared describing the cerebral findings in man and in experimental animals in hypoglycemia. A review of these reports failed to reveal any consistent lesions. The findings in both the human and the experimental material varied from what were apparently normal conditions of the brain (Thalhimer and Murphy;[6] Anderson;[7] Smith and Seibel;[8] Membrez and Razdolsky;[9] Barnard;[10] Bowen and Beck[11]) to most extensive changes in the cells, fibers and vessels (Wohlwill;[12]

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1. Womack, N. A.; Gnagi, W. B., Jr., and Graham, E. A.: J. A. M. A. **97**:831-836, 1931.
2. McClenahan, W. U., and Norris, G. W.: Am. J. M. Sc. **177**:93-97, 1929.
3. Carr, A. D., and others: J. A. M. A. **96**:1363-1367, 1931.
4. Nielson, J. H., and Eggleston, E. L.: J. A. M. A. **94**:860-862, 1930.
5. Cammidge, P. J.: Brit. M. J. **2**:738-741, 1928.
6. Thalhimer, W., and Murphy, F. D.: J. A. M. A. **91**:89-91, 1928.
7. Anderson, H. B.: Am. J. M. Sc. **180**:71-79, 1930.
8. Smith, M. G., and Seibel, M. S.: Am. J. Path. **7**:723-739, 1931.
9. Membrez, G., and Razdolsky, I.: Zentralbl. f. Chir. **58**:1627-1631, 1931.
10. Barnard, W. G.: J. Path. & Bact. **35**:929-932, 1932
11. Bowen, B. D., and Beck, G.: Ann. Int. Med. **6**:1412-1425, 1933.
12. Wohlwill, F.: Klin. Wchnschr. **7**:344-346, 1928.

Terbrüggen;[13] Bodechtel;[14] McClenahan and Norris[2]). Damage of the nerve cells appeared to be the most common finding. It was diffuse, involving all the cortical layers, and consisted of paling of the entire cell. The injured ganglion cells showed little or no Nissl granulation, and the cell bodies were frequently fragmented, shrunken and occasionally infiltrated with fat droplets. Proliferative changes in the glial elements were reported only by Wohlwill, while both Wohlwill and Bodechtel described moderate swelling of the axis-cylinders. McClenahan and Norris observed slight perivascular round cell infiltration, which they interpreted as a sign of mild cerebral irritation or inflammation.

Experimental studies on the cerebral changes in hypoglycemia have been reported by Schereschewsky, Mogilnitzky and Gorjaewa,[15] by Stief and Tokay[16] and by Grayzel.[17] These investigators produced hypoglycemic convulsions in dogs and rabbits through the administration of insulin. Stief and Tokay and Grayzel described changes occurring only in the cellular structures, which showed definite signs of degeneration with partial or complete chromatolysis, vacuolation of the protoplasm, fragmentation of the cell processes and pyknosis of the nuclei. Grayzel believed that the more prolonged and severe the convulsions the more extensive was the cell damage. Schereschewsky and his co-workers reported that numerous petechiae were scattered throughout the cerebral hemispheres of their hypoglycemic animals. They believed that many of the clinical symptoms in the animals were caused by the petechiae in the brain tissue.

From a review of the reported studies it becomes readily apparent that the cerebral lesions described in hypoglycemia are variable. Even studies on the animal are as yet too few to aid materially in an analysis of the organic lesions of the central nervous system caused by a hypoglycemic condition. Because of the paucity of available observations in this condition, a report of our findings in the central nervous system in three patients who died of hypoglycemia seems permissible.

REPORT OF CASES

CASE 1.—A white man aged 29 years was first seen in January 1934, when he complained that for a year he had suffered from transient attacks of irritability, restlessness, fatigue and sweating. These attacks were usually brought on by exertion and relieved by rest. As they increased in frequency they were accom-

13. Terbrüggen, A.: Beitr. z. path. Anat. u. z. allg. Path. **88**:37-59, 1931.

14. Bodechtel, G.: Deutsches Arch. f. klin. Med. **175**:188-201, 1933.

15. Schereschewsky, N. A.; Mogilnitzky, B. N., and Gorjaewa, A. W.: Endokrinologie **5**:204-224, 1929.

16. Stief, S., and Tokay, L.: Ztschr. f. d. ges. Neurol. u. Psychiat. **139**: 434-461, 1932.

17. Grayzel, D. M.: Arch. Int. Med. **54**:694-701, 1934.

panied by mental confusion of varying degree. At times they were manifest merely in momentary blurring of vision and slight faintness or vertigo.

In April 1933, while working on a tractor on his farm, he suddenly lost consciousness. He was immediately removed to a clinic, where a tumor of the brain was suspected at first. The blood sugar was found to be 46 mg. per hundred cubic centimeters. On the intravenous administration of dextrose the patient promptly regained consciousnes. A diagnosis of hypoglycemia was made, and in June an exploration of the pancreas was made. No gross or microscopic lesion was found to account for the patient's attacks. He was discharged and advised to eat at frequent intervals. In spite of this regimen he continued to have one or two attacks of unconsciousness a week. These attacks usually occurred about three hours after meals and lasted from a few minutes to many hours. The patient was often forewarned of an attack by a feeling of restlessness, sweating and blurring of vision and could frequently abort it by taking food.

He was admitted to the Minnesota General Hospital in a semiconscious state. The blood sugar on admission was 30 mg. per hundred cubic centimeters. During his stay in the hospital he experienced many hypoglycemic attacks. Although these attacks varied in severity and duration, they frequently followed a more or less uniform sequence of events. At the onset he became irritable and restless, and the muscles of his hands and face twitched. Although as a rule he was an exceptionally well behaved patient, during these periods he often became silly, annoyed the nursing staff and frequently had outbursts of laughing and shouting. He rapidly became disoriented and drowsy and perspired freely. At times he became violent, threw himself out of bed and resisted or struck people near him. As these attacks increased in severity, definite convulsive seizures of a tonic and clonic nature developed, his pupils became dilated and fixed, his face flushed, and he lapsed into a deep coma. His deep reflexes were usually hyperactive but were variable, and the Babinski sign was present bilaterally. These attacks varied in frequency, often occurring five times daily, and then again not occurring for days. Deep coma could usually be prevented by administering concentrated dextrose only. If deep coma did occur, it was often days before the patient recovered completely.

This patient was observed closely from January 1934 until May 1935. Numerous attempts were made to discover the cause of his attacks and to find some means of preventing them altogether. Various diets and innumerable types of drug therapy were tried, with very little benefit. These studies will be discussed in more detail in another publication. Exploration was resorted to twice. The patient failed to survive the last operation.

Permission for complete necropsy was obtained. There were no significant somatic changes with the exception of moderate bilateral bronchopneumonia and a hematoma of moderate size at the head of the pancreas. Microscopic studies were made of the pituitary, adrenal and thyroid gland, pancreas and testes without detecting anything particularly abnormal.

The scalp and calvarium were normal. No gross evidence of cerebral neoplasm, edema or hemorrhage was found. The brain was fixed in solution of formaldehyde, U. S. P. (1:10), and sections were taken from representative regions throughout the cerebral cortex and white substance and from the basal nuclei, midbrain, pons, medulla and cerebellum. These sections were stained with the hematoxylin-eosin, azocarmine, Nissl, fat-staining and silver impregnation methods.

The ganglion cells revealed no apparent decrease in number in any of the sections studied, although they did show numerous variations from the normal, the cytoplasm being the most affected. One of the mildest changes

was paling of the cytoplasm, which stained lighter and failed to reveal the details ordinarily seen; in the more advanced changes there resulted complete homogeneity of the protoplasm. The dendrites frequently were fragmented and persisted as detached processes surrounding the cell body. When they were entirely absent, the cell assumed a rounded appearance, which together with the cytoplasmic shrinkage resulted in marked variation in the size and shape of the cell. In no case was the cytoplasm entirely absent. Swelling of the cell body was also rarely seen.

The nuclei were much less involved, although they, too, showed moderate changes, especially in their staining properties. As a rule, the nuclear membrane was well outlined, although in scattered cells it was partially or completely obliterated, giving the nucleus an irregular, indistinct appearance. Some nuclei were fragmented and others pyknotic. In every cell the nucleolus was intact. Complete fading out of the nerve cells to form ghost cells or complete destruction of these cells was not observed. Staining by the Nissl method revealed an almost complete loss of Nissl substance in all the ganglion cells. There was no evidence of neuronophagia in spite of the apparent injury to the nerve cells. Satellites were absent. Fat stains revealed no fat particles within any of the cellular elements.

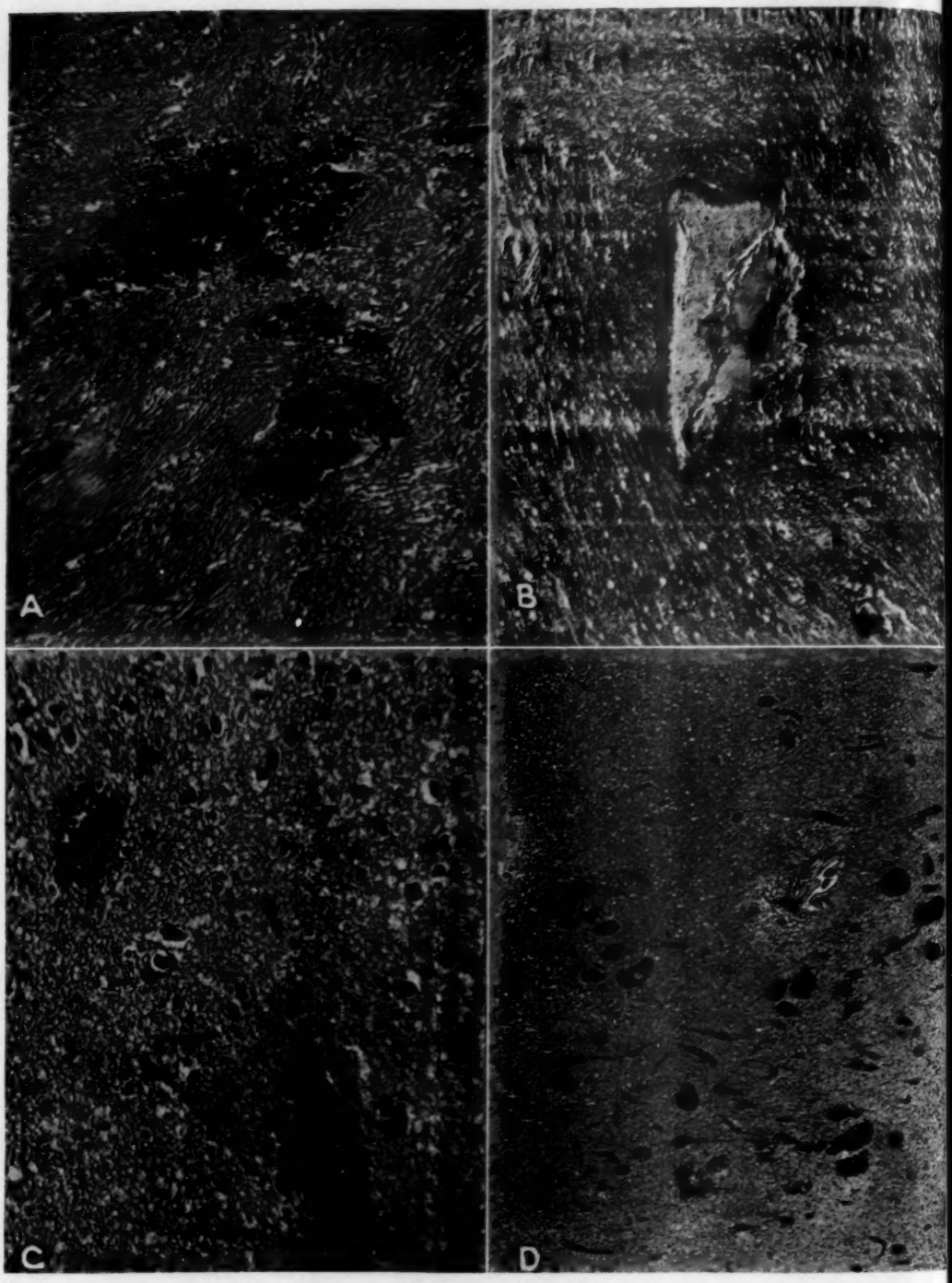
These alterations within the ganglion cells occurred irregularly throughout the brain and were not characteristic or specific for any particular location. Moreover, in each individual section there appeared to be no particular selection for any cortical cell layer. In general, the altered ganglion cells were present throughout the brain substance, intermixed with the numerous apparently normal cells; all cells, however, showed chromotolysis of some degree.

The Purkinje cells of the cerebellum, as well as the nerve cells within the basal nuclei, showed alterations similar to those described.

An almost constant feature of the sections examined was the presence of numerous old and new hemorrhages, which varied from small discrete extravasations scattered irregularly throughout the brain tissue to tiny perivascular bleedings. These hemorrhages were rarely extensive and did not seem to cause injury to the surrounding brain tissue. Most of the petechiae assumed a definite perivascular arrangement, and in those in which the vessel was not visible the solid rounded appearance of the hemorrhage strongly suggested a vascular origin (figure, *A*). The cerebral blood vessels, including those that were situated at the centers of hemorrhages, appeared normal, and the vascular injury that permitted these extravasations of red cells could be detected only rarely. An occasional hemorrhage, however, did contain at its center a ruptured vessel with the erythrocytes streaming out into the perivascular space.

Along with these fresh extravasations there was also evidence of older hemorrhages. These appeared as discrete and often perivascular accumulations of erythrocytes in which the cells had already undergone partial or complete hemolysis and therefore failed to take the ordinary cell stains. They usually appeared as a homogeneous mass, which occasionally revealed under high magnification the faint outlines of some of the erythrocytes that had not as yet undergone complete lysis. An occasional blood vessel was accompanied by a perivascular accumulation of similar hemolyzed and partially destroyed red cells. Also, certain of these vessels were filled with a hyaline material which revealed no sign of any cell structure (figure, *B*).

Cellular reactions to these old or new hemorrhages were not observed, although moderately increased density of the glia could occasionally be detected about a few of the old hemorrhages. It appears that small hemorrhages do not



EXPLANATION OF FIGURE

A, petechiae observed in case 1. These hemorrhages are fairly discrete and suggest a vascular origin.

B, photomicrograph of an old hemorrhage. This hemorrhage appears as a homogeneous mass, the erythrocytes having undergone complete lysis.

C, photomicrograph of a section of the brain in case 2. Note the extensive shrinking of the ganglion cells. The detailed structure of these cells cannot be made out. The hemorrhages are solid, small but distinctly localized.

D, section through the left occipital white substance in case 3. This low power view reveals the grouping of the petechiae and their solid discrete appearance.

stimulate the microglia or astrocytes unless some nerve tissue is destroyed. The absence of such cellular changes seems to indicate, therefore, that the petechiae observed in this brain tissue had not resulted in damage of any considerable degree.

Most of the smaller cerebral vessels were distended with blood, indicating a fairly marked vascular congestion. Moderate distention of the perivascular spaces was also observed. In many sections the edema extended beyond the spaces into the surrounding brain tissue.

Another histologic alteration consisted in small cavities involving both the gray and the white substance. These were observed most frequently in the occipital region and in the basal nuclei. They were small, frequently no larger than the average petechia. The cavities were usually empty, although occasionally a few hemolyzed red cells could be found still adherent to their walls. The walls of these cystic areas were smooth and sharply outlined, and consisted of fairly dense glial fibers that gradually blended into the surrounding brain tissue. The origin and significance of these cavities are not certain, although their appearance strongly suggests that they might represent the remains of an old hemorrhage that had undergone hemolysis. The presence of the glial walls about them is, of course, evidence of their antemortem origin.

No changes were detectable in the myelin or in the axis-cylinders in the sections studied.

In case 1 the histologic studies revealed extensive changes in the ganglion cells as well as many old and new hemorrhages scattered throughout the brain substance. It appears certain that the petechiae occurred before death. This belief is strengthened by the observation of numerous old hemorrhages, of occasional vascular thrombi and of glia-lined cavitations. Evidence that the cell changes occurred ante mortem, however, is not so strong. The fact that the cellular involvement was diffuse, the chromatolysis generalized and neuronophagia and satellitosis completely absent cause one to suspect that the alterations were not present before the death of the patient.

Case 2.—A white man aged 56 years was first observed in December 1933 because of recurrent attacks of so-called nervousness over a period of five years. During these attacks he became greatly excited and showed marked trembling of his head and hands. At times he became morose and depressed, refusing to eat or to obey orders. He was observed casually over a period of about a year and a half. During this time it was noticed that his attacks usually occurred in the morning before he had had breakfast, and that they varied markedly in frequency and intensity. Frequently they manifested themselves merely as severe headache and slight trembling of the head and hands, while at other times they appeared as an emotional upset, the patient often becoming unruly, noisy and disobedient. At such times he frequently refused to talk or eat his meals. Whenever he was forced to take food, the symptoms slowly disappeared and he again became apparently normal in every respect. Convulsions did not occur until the last month of his illness. Although the attacks gradually increased in frequency, they never occurred more than twice weekly.

During the last few months of his illness the patient's condition in these attacks varied from stupor which persisted for hours to acute mania, during which he would become very noisy and would laugh, shout and tear his clothes

and bed covers. The periods of stupor gradually increased in frequency and duration. His last attack occurred about three days before death and was characterized by excessive perspiration, twitching of the head and hands, marked convulsive seizures and finally deep stupor, from which he did not recover.

A complete necropsy was made nine hours after death. The thoracic cavity and all its contents were normal. The abdominal viscera were also apparently normal with the exception of the pancreas, which contained in the region of its head an encapsulated tumor 3.5 by 4.5 cm. Histologic sections of this tumor revealed definite adenoma; we were not able to determine whether it was composed of island, acinar or duct tissue. The rest of the pancreas, its ducts and the common bile ducts were uninvolved.

The brain appeared normal on external inspection. The vessels at its base revealed a slight amount of patchy sclerosis. The spinal cord was normal grossly. Sections were taken from numerous areas throughout the brain and from the spinal cord at various levels and were stained similarly to those in the previous case.

Histologic studies of the central nervous system revealed changes somewhat similar to those already described in case 1. The alterations in the ganglion cells were, however, more severe. The cytoplasm was the most severely damaged. In many cells it was very pale and homogeneous in appearance; in others it was almost entirely absent, leaving only the nucleus situated in a large clear area; while in still others an irregular layer of cytoplasmic substance still surrounded the apparently normal nucleus. The cell processes were uniformly absent. This absence of the dendritic processes together with the cytoplasm degeneration usually gave to the nerve cells an irregular rounded, shrunken appearance, with a clear space separating each cell from the surrounding brain tissue (figure, *C*). There was complete disappearance of the Nissl granules in all the cells studied. The nuclei were much less involved and were as a rule well outlined and fairly intact. Often they stained darker than normal, so that many of their structural details were obscured; but none appeared shrunken or fragmented. The nucleoli were usually present and normal.

Within the basal ganglions the nerve cells assumed a slightly different appearance. Many of the cells were swollen; their cytoplasm was homogeneous and glossy, and they often stained so faintly that they presented the appearance of the so-called ghost cells.

There was no striking reduction in the actual number of nerve cells within the brain and spinal cord, even though scattered cells had undergone complete degeneration. The nerve cell injury was not selective of any part of the nervous system; it seemed to involve equally all regions and all cortical layers. No neuronophagia or satellitosis was observed in spite of the apparently severe cellular damage.

The hemorrhagic lesions within this brain, in comparison with the nerve cell injury, were much less marked. There was extensive vascular congestion, most of the smaller vessels, from the tiny capillaries to the moderate-sized arterioles, being distended with red cells. Petechial and perivascular hemorrhages, although present, were much less frequent than in the previous case. The hemorrhages were usually solid, small and scattered irregularly throughout the brain tissue (figure, *C*). The blood vessels appeared normal. An occasional old hemorrhage made up of hemolyzed red cells was found in a few sections.

Some of the blood vessels were surrounded by numerous cells containing very small dark fairly regular nuclei. A few polymorphonuclears were intermixed with the mononuclear cells.

A few small circumscribed areas of hyalin-like tissue were observed within some of the sections. These were often as large as a good-sized blood vessel and revealed no structural detail in spite of careful study. Some of these homogeneous areas showed beginning cavitation, but no cellular or glial reaction was detected within the surrounding brain tissue.

The sections of the spinal cord were normal with the exception of extensive vascular congestion and occasional petechial and perivascular hemorrhage.

In case 2 the histologic observations were somewhat similar to those in case 1. The changes in the nerve cells were extensive and diffuse. Their character and distribution again strongly suggest postmortem change. The hemorrhages, both old and new, were not numerous. It is possible that the infrequency of the convulsive element in this patient's attacks may account for the relative paucity of the petechial and perivascular hemorrhages.

CASE 3.—A white woman aged 27 years was brought into the hospital in a semistuporous condition a few days before her death. She had had spells of nervousness for the past four years, and there was a moderate but persistent loss of weight. During the past few weeks the patient complained of abdominal pain occurring about half an hour after meals and accompanied by abdominal distention. The abdominal distention was usually relieved by laxatives.

Suddenly, twenty-four hours before admission, the patient became stuporous, responding only with difficulty. On her entrance to the hospital physical examination revealed an emaciated woman in a semiconscious state. The results of the physical examination were entirely negative; no abdominal masses were palpated. The temperature was 98 F., the pulse rate 98, and the respiratory rate 46. The spinal fluid on examination was normal with the exception of the sugar, which was found to be 22 mg. per hundred cubic centimeters. Studies of the patient's blood and urine showed nothing of note.

After having been in the hospital about twelve hours, the patient presented the Babinski sign bilaterally. Her deep reflexes all became hyperactive, and examination of the fundi revealed a slight edema of the nasal side of each nerve disk. The patient died without regaining consciousness.

A complete postmortem examination was performed four hours after death. When the peritoneal cavity was opened, a large mass was seen extending from the duodenum to the spleen. This mass extended into the mesentery over the pancreas. The pancreas was extremely firm and irregular. In its midportion there was a firm elevated tumor 6 by 4 cm. The tumor was poorly circumscribed except on its anterior surface, which was covered with a firm layer of fibrous tissue. It extended to the tail of the pancreas and invaded the medial surface of the spleen. The blood sugar determined on a sample of blood obtained at the autopsy was 45 mg. per hundred cubic centimeters.

The scalp, calvarium and meninges were normal. Externally the brain revealed nothing of note. Serial coronal sections revealed no architectural or other abnormality except petechial hemorrhages in a small area within the white substance of the left occipital lobe. Histologic studies were made of representative regions by the various tinctorial methods listed.

The ganglion cells showed alterations similar to those described in the previous cases. These changes varied from paling to marked shrinking and fragmentation of the cytoplasm. Many of the ganglion cells throughout the brain

seemed to have undergone striking alterations in their normal staining properties. These cells assumed varying histologic appearances. Some seemed to absorb only the hematoxylin or basic stain, which was deposited uniformly throughout the cells, giving them the appearance of irregular deep-purple masses in which no nucleolus, nucleus or other cell detail could be detected. Other cells seemed to have become altered in an entirely different manner. They appeared to have lost their ability to absorb any stain; at best they absorbed only very little of the eosin or acid stain. These cells naturally stained very faintly and were hard to outline, the cytoplasm often merging imperceptibly into the surrounding brain tissue. In many only a most careful study revealed any sign of the actual cell boundary.

Chromatolysis was complete in all the cells studied. The cellular alterations appeared to be distributed irregularly throughout all the sections.

Scattered throughout the brain were numerous hemorrhages that were not visible on gross examination. These microscopic petechiae were mostly fresh and of a solid type, although they were not so well circumscribed as those found in the previous cases. They tended to be irregular and somewhat diffuse, spreading into the surrounding brain tissue. Some of the larger hemorrhages undoubtedly caused some injury to the nerve fibers. No cellular reaction, however, was detected about these hemorrhages; but this was not surprising since the damage was too recent to produce any reaction on the part of either microglia or astrocytes.

Most of the hemorrhages were, as in the previous cases, scattered irregularly throughout the brain tissue without any indication of localization. In the left occipital white substance there occurred a moderate-sized group of hemorrhages that were definitely grouped and large enough to be detected on gross examination. In this area there was an extensive distention of almost all the blood vessels with erythrocytes. These petechiae were of the ball type, well localized, showing no indication of fusing with one another to form larger more destructive lesions (figure, *D*).

In case 3 a group of petechiae was visible grossly in the left occipital region. Histologic study revealed extensive changes of the ganglion cells, the most marked alteration being the inability of the cells to stain with the various routine stains. The nature of this change seems quite characteristic of a postmortem alteration. The hemorrhages as a whole were similar to those in the previous cases. However, there did occur in a large localized region petechiae and probable injury of brain tissue, which might have resulted in some permanent functional damage had the patient lived long enough for such changes to occur.

EXPERIMENTS ON ANIMALS

In all of the three cases described, some alterations were observed in the nerve cells. Their general nature strongly suggested postmortem destruction rather than change that had taken place during life. However, there still remained the possibility that a certain amount of cell damage had occurred before the patient's death and that the evidences of this damage had become obliterated by the more severe postmortem damage. Since it usually is quite impossible to obtain human brain

material early enough after death to eliminate entirely the suspicion of autolysis, it was decided to attempt to reproduce hypoglycemic convulsions in rabbits and then study the brain tissue.

Six rabbits were selected for this experiment. Severe hypoglycemic convulsions were produced by intravenous injections of from 8 to 70 units of insulin. Each animal had been starved for twenty-four hours prior to the injection of insulin. The convulsions were allowed to continue for varying lengths of time up to one hour and were terminated by intravenous injections of 10 cc. of 50 per cent dextrose solution. The hypoglycemic attacks were produced once or twice each week for from six to eight weeks. In the interval between convulsions the animals appeared perfectly normal. In most of the rabbits the convulsions were very severe, but recovery always occurred promptly on the administration of dextrose. Four rabbits were allowed to recover and live for at least five days following the last hypoglycemic attack before they were put to death. This procedure was followed in order to allow sufficient time for cellular changes to occur. Two rabbits were allowed to die in severe hypoglycemia in order that we might study any possible acute changes that had occurred.

Since these experiments were undertaken merely in conjunction with the study of the human material and not as an extensive independent investigation, no attempt will be made to give a lengthy description of each rabbit brain. It must be kept in mind that the gradations between normal and abnormal ganglion cells are often varied and subtle. It therefore becomes essential to keep the normal structure constantly in mind in order to assure accuracy in the interpretation of supposedly abnormal findings. Hence a careful study of the brains of normal rabbits as well as of those of the hypoglycemic animals was made, and a brief description of their cells seems indicated.

The ganglion cells in a normal rabbit present a curious picture when stained with the routine hematoxylin and eosin stain. They stain irregularly, some cells being deeply stained and others lightly, so that the entire section presents an irregular splotchy appearance on superficial examination. In most instances the entire cell stains readily, the cytoplasm appearing much lighter than the rest of the cell. The cytoplasmic granules are extremely large and stain deeply with hematoxylin. These deeply staining granules within the cell body account for the apparently overstained appearance of some of the cells. The nucleus is regular and contains a definite nucleolus. The nucleus and cytoplasmic granules stain with about equal intensity, so that when the latter are numerous the nuclear outline is often hard to make out, and on superficial examination such a cell may appear somewhat pyknotic and seem to be undergoing degenerative changes. Actually, on high power examination, each cellular detail is clearly normal. Cell processes are not numerous and frequently are not seen, the cell being left with an irregular contour. The interstitial cells of a rabbit's brain stain lightly and contain large vesicular nuclei with finely granular chromatin material and sharply outlined nuclear borders.

By comparison with this normal histologic picture very little change could be found in the brains of the hypoglycemic animals. No striking cell lesions were visible. It is true that many of the cells appeared hyperchromatic and occasionally even shrunken, but careful study indicated that all the cell elements were intact and that their differentiation had been made difficult by the extremely large, deeply staining cytoplasmic granules, which decreased the contrast between the cytoplasm and the nucleus. The shrunken appearance of the occasional nerve cell is easily explained by the manner in which the cell was cut, since all the cell constituents were apparent and intact. Only an occasional petechia was observed within the hypoglycemic brains. Some extravasations of erythrocytes into the meninges were seen. Mild chromatolysis was observed in the ganglion cells of the two rabbits that were allowed to die in severe hypoglycemia. However, this acute change was by no means prominent, involving only a few scattered groups of cells.

In general, therefore, when compared with the normal brains, the brains of rabbits in which repeated hypoglycemic attacks had been produced revealed no cell alteration of pathologic significance and only an occasional hemorrhage into the brain or meninges. Our experimental observations, therefore, would seem to add weight to our impression that in the human cases the cellular alterations were most likely of a postmortem nature.

GENERAL COMMENT

The detailed histologic studies of the central nervous system in these cases of hypoglycemia bring to light certain interesting features that warrant emphasis. In all our cases there were revealed extensive and widespread alterations of the ganglion cells, both the cytoplasm and the nucleus being involved. The degree of destruction varied in each section and in each case studied. There apparently was no localization in any nerve cell layer or even in any part of the brain; the injury was usually disseminated. Cell changes similar to these can be observed in normal brains which are removed at a corresponding length of time after death. The entire character of these findings strongly suggests postmortem change; therefore, in spite of the fact that other authors have described cell alterations in brains of hypoglycemic persons, one hesitates to ascribe too much significance to this finding in human brains obtained for study hours after death.

There remains the distinct possibility, however, that some antemortem cell damage did occur and was masked by the changes resulting after death. In order to verify this point, experiments on animals were undertaken in an attempt to obtain absolutely fresh nerve tissue. It was readily apparent from these experiments that the cellular altera-

tions, even those found after many severe hypoglycemic attacks, were negligible. The ganglion cells were normal in size, shape and structure and failed to reveal the extensive changes seen in the human material. This finding seemed to strengthen our original impression that the cell changes which we observed in man probably had occurred post mortem and were of no significance.

What, then, can we assume is the explanation of the marked neurologic manifestations in these patients with so few histologic changes in the nervous system? It appears that the nutritive requirement necessary to maintain the life of the nerve cell is much smaller than that necessary to maintain its normal function. In severe hypoglycemia cerebral function may become depressed to such a degree and in such an irregular manner that variant neurologic manifestations occur. However, in spite of the extremely decreased cerebral function, ample nutrition to sustain the life and structure of the brain cells may still be available. On the restoration of the normal blood sugar the cells may be able to regain promptly their normal function. Only such a hypothesis can explain the prompt and complete recoveries after repeated severe attacks.

A final feature of study is the presence of tiny hemorrhages scattered irregularly throughout the brain. In most sections, the size and distribution of the petechiae are of such a nature that no apparent injury is done to the surrounding tissue, the erythrocytes merely pushing the cerebral fibers apart without causing them to rupture. Little or no alteration in the normal cerebral function would be expected to occur as a result of these tiny petechiae. However, there remains the likelihood that during a severe convulsive seizure the resulting hemorrhages may become confluent with resultant injury to the surrounding brain tissue by pressure or actual destruction. Such a process occurring within an important brain center can well give rise to temporary or permanent neurologic manifestations long after the restoration of the rest of the brain to normal function.

SUMMARY

In detailed histologic studies of the cerebral changes in three cases of hypoglycemia, numerous new and old hemorrhages were found scattered irregularly throughout the three brains. These seemed most numerous in the brains of the patients who had the most severe convulsive seizures. The ganglion cell changes in the brains were due entirely to postmortem alterations.

The brains of rabbits in which repeated hypoglycemic convulsions were produced showed no cell alteration of pathologic significance.

THE BLOOD OF FEMALE MICE (BREEDERS) OF CANCER-SUSCEPTIBLE (A) AND CANCER-RESISTANT (CBA) STRAINS

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Precipitation tests made on the blood of cancerous and noncancerous strains of mice have been reported by Strong and Werner [1] and Strong; [2] from the results of their studies, made under various conditions, these authors have indicated that there appears to be an association between susceptibility to spontaneous mammary tumors and a low volume of a precipitate obtained from whole blood after treatment with trichloro-acetic acid. As is well known, such a precipitate contains all the nitrogenous constituents of whole blood except the nonprotein nitrogen. In this mixture of all the blood proteins, hemoglobin is by far the largest single component, amounting in the human species to about 83 per cent of the total protein contained in whole blood. The possible importance of variations in other protein fractions of the precipitate should not be overlooked, however, and may need to be investigated later.

The portion of the present study dealing with blood precipitate was undertaken in order to discover whether the volume obtained from the cancer-susceptible (A) strain of mice would continue to be lower than that secured from the cancer-resistant (CBA) strain if the experimental conditions and quantitative technic employed were more exacting than those which had been used in the preliminary study reported from another laboratory. In addition to determining the volumes of trichloro-acetic acid precipitate obtained from whole blood we also studied the concentration of hemoglobin in an effort to measure more exactly the variable component in the blood precipitate, for variations in the concentration of hemoglobin, the largest protein constituent of the precipitate, might easily account for the differences observed in the volumes of blood precipitate.[1]

The studies were aided by grants from the Josiah Macy Jr. Foundation.

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1. Strong, L. C., and Werner, T. H.: Am. J. Cancer **26**:767, 1936.
2. Strong, L. C.: Am. J. Cancer **27**:115, 118 and 500, 1936.

APPARATUS AND METHODS

Hemocytometers, Newcomer hemoglobin pipets, and centrifuge tubes specially designed and made for this study were all calibrated by the method described by Francis.[3] The special centrifuge tube was graduated to 0.001 cc.; hence the bore of the stem was much less than that of the Hopkins tube previously used.[1] Quantitative accuracy in reading the volume of precipitate was greatly enhanced by the capillary character of the graduated portion of this special tube: The bore was so narrow that a 19 gage hypodermic needle was the largest size that could pass into the stem. To remove the firmly packed precipitate from the tube, it was necessary to use suction applied through the hypodermic needle.

The method of precipitation employed by Strong and Werner,[1] i. e., laking blood with acetic acid and developing a precipitate by the addition of trichloroacetic acid, was used with modifications. The details of the method were first studied experimentally, however, in order to establish the optimum conditions with regard to the volumes of the two acids and the time of standing following the addition of each acid which would produce complete precipitation of the proteins from the acetic acid solution of the blood. Pooled blood was employed in part of this preliminary study.

In order to prevent precipitate from lodging on the shoulders of the special tubes during centrifugation, a condition which had given rise to difficulty in using the Hopkins tubes,[1] the new ones were designed so that the large end was reduced very gradually to the small size required for the stem (streamlined); nevertheless, precipitates sometimes collected on the gradually sloping shoulders of these tubes. After experimenting with various combinations of periods of time and rates of speed, the one which gave the best results was adopted.

Because three centrifuges had to be employed to provide sufficient space for the work, it was imperative to secure from them results which would be comparable. The speed and the time required for centrifugation were therefore studied with pooled blood sufficient to furnish triplicate series (one for each centrifuge) of four determinations each. Within the limits of error of the method, consistent and interchangeable results were obtained from the pooled blood when the precipitate became so tightly packed that its volume could not be lessened either by longer centrifugation or by using more speed. The conditions which produced comparable results with pooled blood thus established for the centrifuges were adhered to throughout the experiment.

In order to increase the accuracy and reliability of the data obtained, it was necessary to read volumes of packed precipitate under exact conditions which, moreover, could be maintained throughout the period of investigation. Hence, factors affecting the accuracy of readings had to be determined and standardized. Such conditions concerned the lighting, position and magnification of the tubes.

To furnish a uniform and suitable light, a box was used provided with a rigid socket for an electric bulb, a pane of frosted glass for the front and a black wooden shutter. By lowering the shutter over the glass, the lighted area could be confined to any satisfactory level. A test tube rack fastened securely in front of the box held the centrifuge tubes. Only one of the holes in this rack was used to support

3. Francis, L. D.: J. Lab. & Clin. Med., to be published.

a tube while readings of the volume of precipitate contained therein were being made. In addition, a hand magnifying lens was held in fixed position on the test tube rack by means of paraffin. In consequence of all this standardization of conditions, readings were made with accuracy and uniformity.

The mice used in this study were breeders of the highly inbred Strong-A [4] and CBA [5] strains. They were all housed alike and throughout the experiment were maintained on the same diet, the oatmeal mixture described by Strong.[6] They were separated from food late in the afternoon but were provided with ample water, and early the following forenoon venous blood was taken from the tail while the animal rested comfortably in a specially designed convenient holder.[7] The period without food preceding the taking of blood from these mice was longer, therefore, than any of the intervals of time used in the earlier studies of blood precipitate.[8] From each mouse at 400 ± 1 days of age four samples of blood were taken: the first was used for the determination of hemoglobin and the others for triplicate precipitate tests. At 440 ± 1 days of age only one sample of blood was taken, for a reading of hemoglobin.

The precipitate was formed by washing 0.0156 cc. of blood contained in a hemocytometer into 2 cc. of a 2 per cent acetic acid solution which had been measured into a special centrifuge tube; after this mixture had stood for ten minutes, 4 cc. of a 5 per cent trichloro-acetic acid solution was run from a buret into each tube of laked blood. The tubes stood for an additional ten minutes and were then submitted to centrifugation at relatively slow speed for a preliminary period of ten minutes. Any precipitate which had failed to collect in the stem of the tube in the form of a continuous mass was stirred into the acid with a platinum wire. Centrifugation was then continued at a very rapid rate for one hour and fifty-five minutes. On completion of the centrifugation, the volumes of precipitate were read under the standard constant conditions described.

The acid-hematin method was used for the determinations of hemoglobin. Readings were made colorimetrically against a hemoglobin disk, which had been standardized previously by the oxygen capacity method and by determinations of the iron. A daily and sometimes more frequent checking was made of the position of the colorimeter with reference to lighting (electric) and to any other factors which might prevent readings from day to day being comparable. The setting of the instrument was determined by taking readings with a "standard" acid-hematin solution of blood in both of the cups. An additional check was afforded by subsequently reading the "standard" solution against the standardized hemoglobin disk before the reading of the unknown bloods was begun.

RESULTS

Results procured from mice representing several groups are presented in tabular form. Data secured indicate that in female mice which had been used as breeders the blood of the A strain is strikingly differ-

4. Strong, L. C.: J. Hered. **27**:21, 1936.
5. Strong, L. C.: Brit. J. Exper. Path. **17**:60, 1936
6. Strong, L. C.: Proc. Soc. Exper. Biol. & Med. **30**:386, 1932.
7. Francis, L. D.: Proc. Soc. Exper. Biol. & Med. **34**:481, 1936.
8. Strong and Werner.[1] Strong.[2]

ent from that of the CBA. Even though overlapping between animals of the two strains occurred in the values obtained for the trichloroacetic acid precipitate of whole blood and for hemoglobin, nevertheless, the significance ratios calculated are sufficiently large to establish the fact that under the conditions of these experiments the blood of the cancer-susceptible mice when compared with that of the cancer-resistant mice yields significantly *lower* values for the precipitate of total proteins and for the concentration of hemoglobin.

The Blood of Cancer-Susceptible (A) and Cancer-Resistant (CBA) Female Mice (Breeders) Maintained on a Mixed Oatmeal Ration *

I. Trichloro-acetic Acid Precipitate of Whole Blood Taken at 400 Days of Age

	Mouse Strain A	Mouse Strain CBA
Number in group	62	57
Mean precipitate, cc.	8.9×10^{-3}	1.01×10^{-2}
Variation from the mean		
Above	3.2×10^{-3}	2.9×10^{-3}
Below	1.7×10^{-3}	2.7×10^{-3}
Standard deviation of means	1.506×10^{-4}	1.964×10^{-4}
Significance ratio	7.2	

II. Concentration of Hemoglobin

Age, days	400		440	
Mouse strain	A	CBA	A	CBA
Number in group	60	51	16	22
Mean hemoglobin, Gm. %	13.4	14.6	13.4	14.3
Variation from the mean				
Above	2.9	7.1	1.3	1.6
Below	4.4	3.9	2.5	3.5
Standard deviation of means	0.2	0.2	0.3	0.3
Significance ratio	5.5		3.3	

* The mice were separated from food eighteen to twenty hours before blood samples were taken.

COMMENT

The difference in the values obtained for the blood precipitate serves to confirm the earlier studies of Strong and Werner [1] reported from another laboratory on the same strains of mice under conditions which varied somewhat from those employed in this investigation. Moreover, the results on precipitate and hemoglobin furnish additional evidence of the existence of physiologic differences between the cancer-susceptible and the cancer-resistant strains of mice.

A low level of blood proteins and of hemoglobin may be a fundamental characteristic of the blood of the A mice at the age investigated, owing perhaps to an aging of the organism which may occur earlier in the A than in the CBA animals; on the other hand, if a significant difference in the hemoglobin concentration of the blood exists through-

out the life of these two strains of mice, the anemic condition of the A in contrast to that of the CBA animals might be attributed to a difference in strain. Furthermore, there is the possibility that the low values observed may be associated with disturbances in the normal physiology of mice having a high susceptibility to spontaneous mammary carcinoma, disturbances which may occur far in advance of the onset of neoplastic growth. It is well known that under certain conditions the hemoglobin level of the blood may be altered by suitable dietary changes. Hence the questions remain to be answered whether the low hemoglobin concentration in the blood of the A mice could be raised by dietary or therapeutic means, and also what the effect on the cancer picture would be of thus improving the oxygen-carrying capacity of the blood of mice having a high susceptibility to malignant tumors, spontaneous in origin.

A study of the concentration of the hemoglobin in the blood of the A and the CBA strains of mice, made at intervals throughout life, has been undertaken; a report will be published shortly. The additional data may aid in explaining some of the questions raised by this investigation.

SUMMARY

A difference was found between the bloods of two inbred strains of female mice which had been used as breeders and which differed from each other in susceptibility to spontaneous mammary tumors: The blood of the cancer-susceptible (A) mice as compared with that of the cancer resistant (CBA) mice had significantly lower levels of hemoglobin and total protein.

LESIONS OF THE NERVOUS SYSTEM OF THE RAT IN VITAMIN B DEFICIENCY

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The clinical syndromes and especially the neuro-anatomic lesions induced by the administration of diets deficient in the vitamin B complex to animals (rats, cats and dogs) are apparently not constant. Most observers agree on the changes induced in the peripheral nerves of some animals as a result of deprivation of the antineuritic component (B_1). Not all, however, agree on the lesions produced in the spinal cord and higher neural centers. Furthermore, a number of observers are of the opinion that the nervous lesions are not the direct result of the vitamin B deficiency in these artificial diets but are due entirely to the inevitable factor of inanition. Certain investigators (Gildea, Kattwinkell and Castle [1]) compare the focal demyelinization induced in the spinal cords of dogs by vitamin B deprivation to the well defined neuropathologic picture of subacute combined degeneration of the human spinal cord (pernicious anemia).

Because of the lack of accord in the previous observations and conclusions an experimental study of vitamin B deficiency in the rat was undertaken. The study includes: (1) rats (*a*) totally and (*b*) subtotally deprived of vitamin B_1; (2) animals (*a*) totally and (*b*) subtotally deprived of vitamins B_1 and B_2; (3) controls fed with (*a*) stock diets and (*b*) artificial diets including adequate vitamin rations; (4) controls subjected to (*a*) total starvation and (*b*) starvation despite adequate vitamin rations. Further experiments on diets deficient in vitamins B_2, A, C, D and E carried out by one of us (Davison) will form the subjects of other presentations.

In this presentation we shall limit ourselves to the lesions of the nervous system and their clinical expressions. We shall attempt to

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A preliminary report of this and other experiments with vitamins was presented at the International Neurological Congress, London, England, on Aug. 2, 1935, and at the International Physiological Congress, Leningrad, Russia, on Aug. 12, 1935.

1. Gildea, E. F.; Kattwinkell, E. E., and Castle, W. B.: New England J. Med. **202**:523, 1930.

discriminate between the lesions of the central nervous system caused by the vitamin deficiency, those due to inanition, and apparent defects due to fixation or staining.

PROCEDURES

Animals.—Twenty-two rats were used. The ages varied between 3 and 4½ months, and the weights varied from 155 to 252 Gm. Four of the animals were from 27 to 36 days old and weighed from 27.5 to 36.5 Gm. One female rat was 6 months old.

Diets.—The diets used were artificial, compounded according to the following formula:

	Gm.
Casein*	180
Cod liver oil	20
Butter fat†	80
Autoclaved yeast‡	150
Corn starch	530
Osborne-Mendel salt mixture	40

* Merck's technical casein—protein, 85, ash, 5, and distilled water, 10 per cent, extracted. The technical casein was purified in the following manner: To 400 Gm. of casein 2 liters of cold 60 per cent alcohol was added and stirred for one-half hour. After standing five and one half hours, this was filtered with suction; then it was washed with 1 liter of 60 per cent alcohol and washed again with 2 liters of 60 per cent alcohol for one-half hour. After standing for eighteen hours, it was filtered and washed with 1 liter of 60 per cent alcohol and finally washed with 1 liter of 90 per cent alcohol, which was finally filtered off.

† The ordinary butter fat was melted at 45 C. and allowed to solidify. Salt and water were separated from the curd, which was washed with water and filtered with a hot water funnel at 45 C.

‡ The baker's yeast was autoclaved at 120 C. for six hours under 15 pounds' (6.8 Kg.) pressure.

In the diets free from both vitamin B_1 (antineuritic component) and B_2 (pellagra-preventing component) the autoclaved yeast was eliminated and the corn starch was increased from 530 to 680 Gm.

A daily ration of 45 Gm. of the food mixture was given each group of three rats. The diet was portioned once a day, and distilled water was provided for all except group 6, which received the stock diet and ordinary water. Baker's yeast was used throughout the experiment.

Later in the experiment, four rats were subjected to starvation. Rat 21 of group 6 (stock diet) was totally starved until it died. Rat 17 of group 5 (yeast adequate) was totally starved until it died. Rat 22 of group 6 was starved but was supplied with a daily ration of bemax and yeast, 1 Gm. of each, until it died. Rat 14 of group 4 (bemax adequate) was starved but was supplied with a daily ration of bemax and yeast, 1 Gm. of each, until it died.

Postmortem Examinations.—Some of the rats were left to die; others were killed as near the point of death as could be determined. In this manner we were able to differentiate between the antemortem and the postmortem changes. The autopsies on all the rats except those that died suddenly during the night were performed immediately.

Sections from the central nervous system (cerebral hemispheres, pons, medulla oblongata, spinal cord, spinal roots and ganglia) and most of the peripheral nerves were fixed in solution of formaldehyde, U. S. P., and stained with the myelin sheath, Spielmeyer, sudan III, Bielschowsky and cresyl violet methods. The myelin sheath and cresyl violet stains were used on sections from material embedded in pyroxylin (celloidin). The Spielmeyer, sudan III and Bielschowsky stains were applied on frozen material. Material from the spinal cords and peripheral nerves was also fixed in Müller's fluid and stained with osmic acid (Marchi's method).

OBSERVATIONS IN RATS ON DIET DEFICIENT IN VITAMIN B_1 (GROUP 1)

Seven rats received the diet deficient in vitamin B_1 uninterruptedly until they died (rats 1 to 7 inclusive).

RAT 1.—A male 3 months and 3 weeks old, weighing 228 Gm., had been kept on the vitamin B_1 deficient diet for one month when there developed weakness of the hind limbs, followed three days later by paralysis of the right hind limb, generalized convulsions, forced rolling movements, hypertonus and nystagmus. The animal died on the forty-first day, weighing 129 Gm.

Autopsy.—Grossly, there was nothing of note. Microscopically, the peripheral nerves of the extremities disclosed disintegration of the myelin in the Marchi, Spielmeyer and sudan III preparations (fig. 1). The most pronounced changes, however, were those noted in the Marchi-stained sections. The white matter of the spinal cord had somewhat of a honeycomb appearance (fig. 2). On careful examination this proved to be an artefact, as no demonstrable changes were noted in the myelin sheaths with the Marchi, myelin sheath or sudan III stains. There were vacuolation and liquefaction of ganglion cells of the mesencephalon and of the anterior horns (fig. 3).

RAT 2.—A male 3 months and 3 weeks old, weighing 187 Gm., after the twenty-fifth day on the B_1 deficient diet showed priapism and catalepsy. On the forty-seventh day there appeared equilibratory disturbances, with occasional convulsions, and on the fiftieth day, extreme asthenia and tonic retractions of the head. The animal died on the fifty-first day, when he weighed 102 Gm.

Autopsy.—Grossly, no abnormalities were found in the central nervous system. Microscopically, with the Marchi, Spielmeyer and sudan III stains, the peripheral nerves disclosed disintegration of the myelin. The fiber tracts of the cord were intact. In the cresyl violet preparation there were vacuolation and chromatolysis of some of the ganglion cells of the cortex and anterior horns; the vacuolation was most pronounced in the latter.

RAT 3.—A male 3 months and 3 weeks old, weighing 198 Gm., presented priapism and catalepsy on the twenty-fifth day, equilibratory disturbances on the thirty-fifth day, paralysis of the hind limbs on the forty-third day and bicycle movements of the extremities and retraction of the head on the forty-fifth day, when the animal died, weighing 97.5 Gm.

Autopsy.—Grossly, there were hemorrhages in the region of the mesencephalon. Microscopically, with all stains the peripheral nerves disclosed disintegration of the myelin. The cerebral vessels were congested. There were numerous hemorrhages within the mesencephalon (fig. 4 *A*). The ganglion cells in the vicinity of these hemorrhages disclosed various pathologic changes, such as chromatolysis, liquefaction necrosis and neuronophagia. Vacuolation of ganglion cells in the mesencephalon and spinal cord was also noted.

RAT 4.—A male 36 days old, weighing 27.5 Gm., had convulsions on the twenty-first day, followed by equilibratory disturbances with the extremities in extension on the next day. The condition remained unchanged, and the animal was found dead on the twenty-seventh day, weighing 28 Gm.

Autopsy.—Grossly, except for congestion of the vessels of the mesencephalon, no abnormalities were encountered. Microscopically, the peripheral nerves, especially those of the right forelimb, disclosed swelling of the single fibers with fat granules in the Marchi preparation. The myelin sheath and fat stains did not show any changes. In the cresyl violet preparation there were congestion of the cerebral vessels and hemorrhages in the mesencephalon with disintegration of

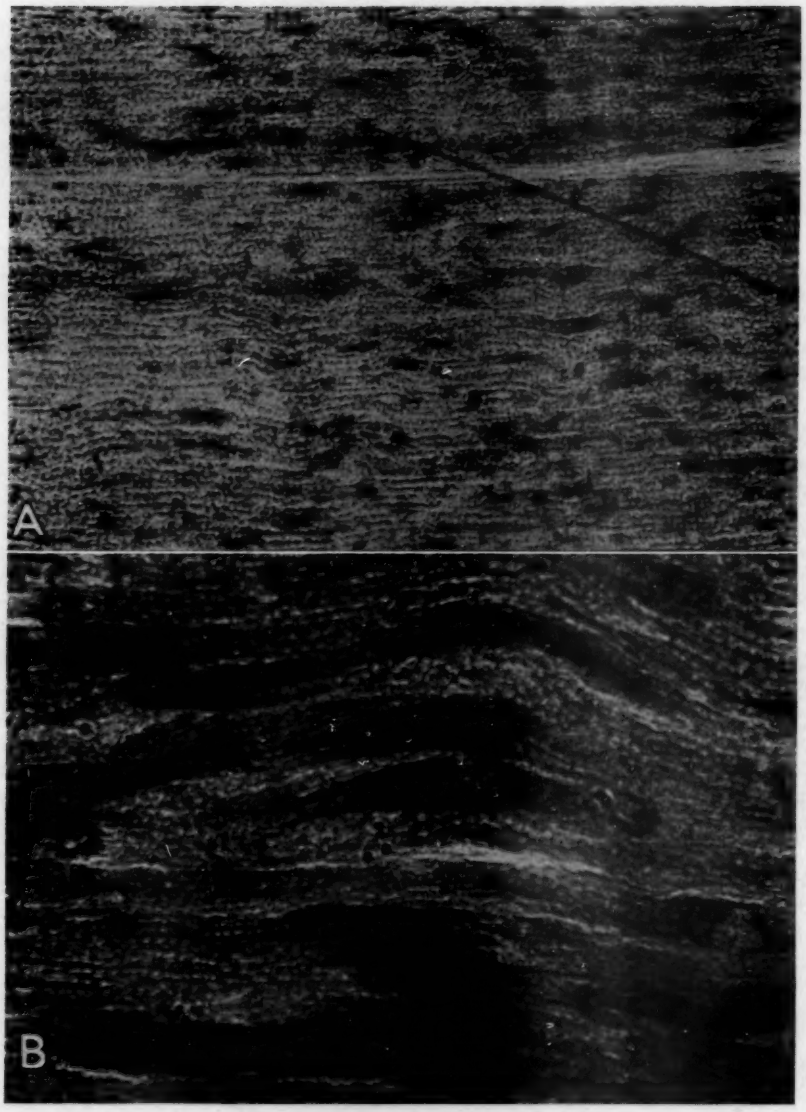

Fig. 1.—*A*, degeneration of a peripheral nerve of a rat deprived of vitamin B_1; Marchi stain; × 240. *B*, destruction of the myelin of a peripheral nerve of a rat deprived of vitamin B_1; myelin sheath stain; × 480.

ganglion cells and proliferation and swelling of glia cells (fig. 4 *B*). Some of the ganglion cells of the rest of the nervous system and of the anterior horns showed vacuolation and coagulation necrosis.

RAT 5.—A male 36 days old, weighing 30 Gm., began to have convulsions and weakness of all the extremities on the twenty-ninth day. Peter's antineuritic extract [1a] was administered (1 cc.) on the thirtieth and thirty-first days, without any improvement in the neurologic picture. The animal died on the thirty-sixth day, weighing 41 Gm.

Autopsy.—Grossly, hemorrhages were observed in the mesencephalon. Microscopically, in the Marchi preparation there was disintegration of the myelin sheaths of the peripheral nerves of the hind limbs. The most marked changes were seen

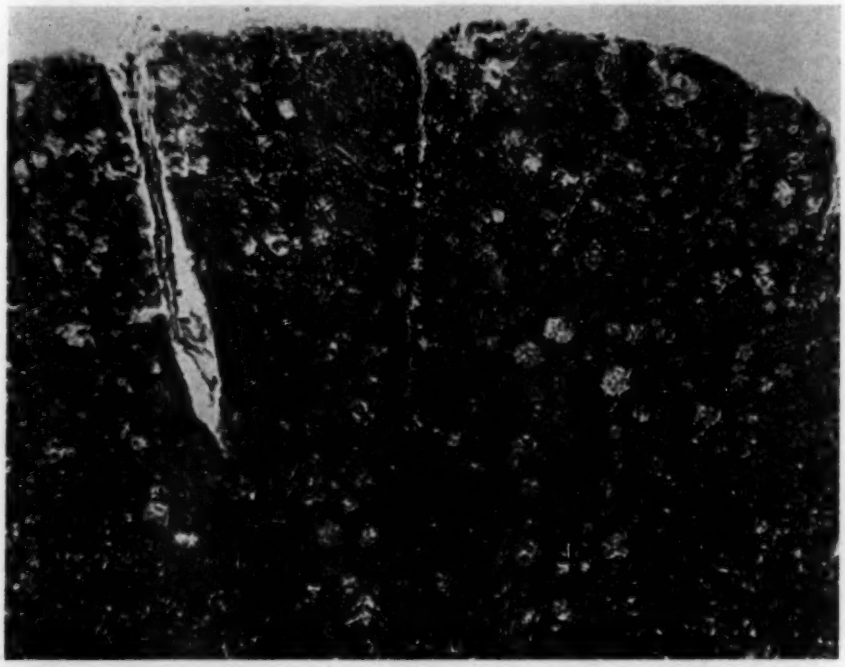

Fig. 2.—Dorsal surface of the spinal cord from a rat deprived of vitamin B_1, showing artefacts and somewhat of a honeycomb appearance; myelin sheath stain; × 100.

in the region of the nodes of Ranvier and lantern chambers (the solitary rings and spirals of Rezzonico). No marked changes were noted in the myelin sheaths in the sections stained with sudan III. In the Bielschowsky preparation there was occasional fragmentation of axis-cylinders. The fiber tracts of the spinal cord showed occasional swelling of single fibers at the periphery. In the cresyl violet preparation the vessels of the cerebrum were found congested. There were numerous hemorrhages within the mesencephalon, with disintegration of ganglion cells and proliferation of glia cells. Some of the anterior horn cells disclosed vacuolation and liquefaction necrosis.

1a. Peters, R. A.: Biochem. J. **18**:862, 1924.

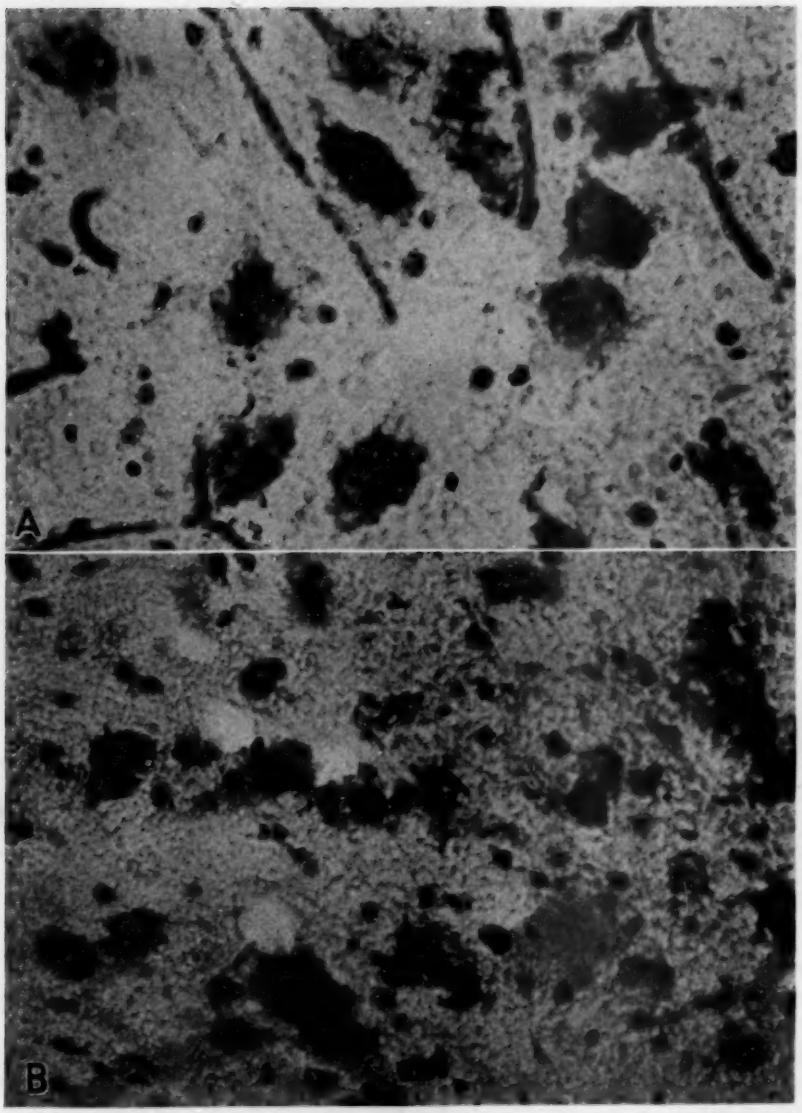

Fig. 3.—Severe cell changes: *A*, liquefaction and vacuolation of ganglion cells of the mesencephalon; *B*, liquefaction and vacuolation of nerve cells of the spinal cord; cresyl violet stain; × 480.

RAT 6.—A female 34 days old, weighing 32 Gm., began to have convulsions and dragging of the hind limbs on the twenty-sixth day. The administration of 1 cc. of Peter's antineuritic extract on two successive days led to a disappearance of

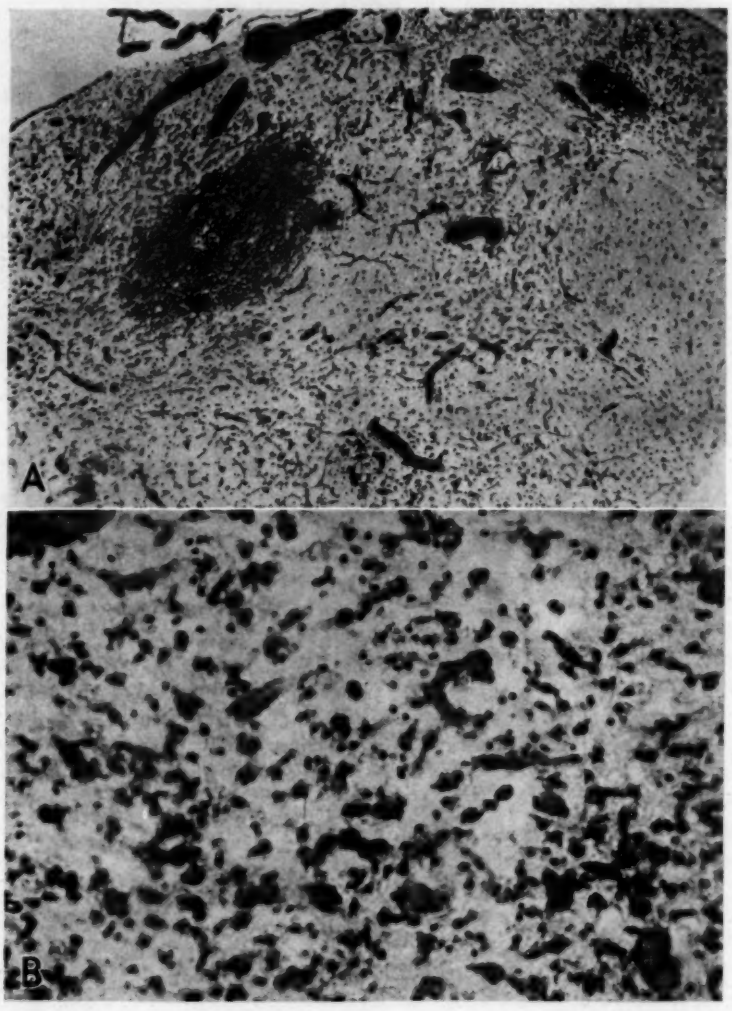

Fig. 4.—*A*, hemorrhages in the region of the mesencephalon; cresyl violet stain; × 60. The animal was deprived of vitamins B_1 and B_2 and had convulsions. *B*, area of softening, hyperemia and proliferation of glia cells of the mesencephalon from a rat deprived of vitamin B_1 and B_2; cresyl violet stain; × 100. This animal suffered from convulsions.

the convulsions and to some improvement in the paralysis of the hind limbs. On the withdrawal of the extract the symptoms recurred, and the animal died on the thirty-ninth day, weighing 38 Gm.

Autopsy.—Grossly, hemorrhages were noted in the region of the mesencephalon. Microscopically, there was slight swelling of single myelin fibers in the peripheral nerves of the hind limbs (Spielmeyer method). Definite disintegration of the myelin sheaths was noted in the Marchi preparations. The cresyl violet preparations disclosed hemorrhages in the mesencephalon with destruction of ganglion cells and proliferation of glia. Vacuolation and liquefaction of ganglion cells in the mesencephalon and anterior horns were also noted.

RAT 7.—A male 34 days old, weighing 36.5 Gm., began to have convulsions, paralysis of the hind limbs and reeling to the right on the thirtieth day. The animal died on the thirty-fifth day, weighing 39 Gm.

Autopsy.—Grossly, there were hemorrhages in the mesencephalon and metencephalon. In the Marchi preparations the peripheral nerves disclosed slight disintegration of the myelin. Slight changes were also noted in the Spielmeyer and sudan III stained sections. In the cresyl violet preparations there were hemorrhages in the mesencephalon and metencephalon with pathologic changes in the glia and ganglion cells. Other nerve cells in the central nervous system and anterior horns disclosed vacuolation and liquefaction necrosis.

OBSERVATIONS IN RATS ON DIET DEFICIENT IN VITAMIN B_1 AND B_2 PLUS VERY SMALL QUANTITIES OF BEMAX (GROUP 2)

After a period of two weeks on a diet deficient in vitamins B_1 and B_2 the three rats in this group were each given 0.5 Gm. of bemax twice a week in the diet.

RAT 8.—A male 3 months and 27 days old, weighing 235 Gm., began to have convulsions, dragging of the hind limbs, tonic retractions of the head and falling to the right on the thirty-sixth day. On the thirty-eighth day there appeared clonic movements of the right foreleg. At death on the same day the animal weighed 115 Gm.

Autopsy.—Grossly, there were no changes. Microscopically, in the Marchi and Spielmeyer preparations the peripheral nerves of the hind limbs disclosed disintegration of the myelin sheaths. Slight changes were also noted in the sudan III preparations. In the sections stained with cresyl violet there were no pathologic changes, except occasional vacuolation of anterior horns.

RAT 9.—A male 3 months and 27 days old, weighing 183 Gm., presented on the fifty-eighth day ataxia of all the extremities and priapism. On the sixtieth day there appeared reeling movements to the right, and on the sixty-second day, tonic retractions of the head. The animal died on the sixty-second day, weighing 102.5 Gm.

Autopsy.—Grossly, there was nothing of note. In the Marchi preparations the peripheral nerves disclosed slight disintegration of the myelin. In the Spielmeyer preparations some of the myelin stained poorly without any change being observable in the fibers. In the cresyl violet preparation vacuolation of the ganglion cells of the mesencephalon and anterior horns was noted.

RAT 10.—A male 3 months and 27 days old, weighing 180 Gm., presented ataxia and weakness of the hind limbs on the thirty-eighth day and died on the forty-first day, weighing 101.5 Gm.

Autopsy.—Grossly, there were no changes. Microscopically, the peripheral nerves of the hind limbs disclosed in the Marchi sections slight disintegration of

the myelin. In the Spielmeyer preparations the fibers stained poorly. In the cresyl violet preparation there was congestion of the cerebral and cerebellar vessels with occasional vacuolation of the ganglion and anterior horn cells.

OBSERVATIONS IN RATS ON DIET DEFICIENT IN VITAMIN B_1 PLUS VERY SMALL QUANTITIES OF YEAST (GROUP 3)

After the three rats had been on the vitamin B_1 deficient diet for two weeks 0.5 Gm. of fresh yeast was added to the diet of each.

RAT 11.—A male 3 months and 27 days old, weighing 179 Gm., began on the thirty-sixth day to show ataxia of the hind limbs, swaying of the head from side to side and tonic retractions of the head. On the thirty-seventh day there occurred clonic convulsions. The animal died on this day, weighing 103 Gm.

Autopsy.—Grossly, there were no abnormalities. The peripheral nerves disclosed in the Marchi preparations slight disintegration of the myelin sheaths and with the Spielmeyer technic demyelinization of the fibers. The spinal cord had somewhat of a honeycomb appearance without any alterations in the myelin sheaths. In the cresyl violet preparation vacuolation of anterior horn cells was noted.

RAT 12.—A female 3 months and 27 days old, weighing 153 Gm., had dragging of the hind limbs on the thirty-first day. Clonic convulsions and convulsive chewing movements appeared on the thirty-second day, when the animal died, weighing 77.5 Gm.

Autopsy.—Grossly, there were subarachnoid hemorrhages over the right cerebral hemisphere and cerebellum. The peripheral nerves of this animal were lost. The vessels of the central nervous system were congested, and the cells of the anterior horns disclosed vacuolation and liquefaction necrosis.

RAT 13.—A female 3 months and 27 days old, weighing 179 Gm., began on the thirty-eighth day to display a broad-based gait, stiffness of the hind limbs and ataxia. On this day it was given 2 Gm. of yeast, with improvement in the neurologic signs. When the yeast was withdrawn the neurologic signs returned. The animal died on the forty-eighth day, weighing 102 Gm.

Autopsy.—Grossly, there were no changes in the nervous system. The peripheral nerves disclosed fragmentation of the myelin sheaths, best observed in the Marchi preparation. The vessels of the cerebrum were congested. There was vacuolation of the ganglion cells of the mesencephalon.

OBSERVATIONS IN RATS ON DIET DEFICIENT IN VITAMIN B_1 PLUS ADEQUATE QUANTITIES OF BEMAX (GROUP 4)

One gram of bemax per rat was added to the diet daily.

RAT 14.—A female 6 months old, weighing 211 Gm., was on this diet for thirty-nine days, during which time the animal constantly gained in weight. After this day the diet, except for 1 Gm. of bemax and 1 Gm. of yeast daily, was discontinued. The animal died three weeks later. There were slight equilibratory disturbances the day the rat died, weighing 119.5 Gm.

Autopsy.—Grossly, there were no changes. Microscopically, the peripheral nerves disclosed disintegration of the myelin sheaths, best observed in the Marchi preparations. The cerebral vessels were congested, and there was marked vacuolation of the ganglion and anterior horn cells.

RAT 15.—A female 3 months and 27 days old, weighing 135 Gm., was kept on this diet for sixty days. Neurologic signs were not present up to the time that it was killed, weighing 192 Gm. There were no gross or microscopic changes in the peripheral nerves or other neural structures.

RAT 16.—A female 3 months and 27 days old, weighing 146 Gm., was kept on this diet for sixty days, without presenting neurologic signs. The rat was killed when weighing 186 Gm. There were no gross or microscopic changes in the peripheral nerves or in the central nervous system.

OBSERVATIONS IN RATS ON DIET DEFICIENT IN VITAMIN B_1 PLUS SUPPOSEDLY ADEQUATE QUANTITIES OF YEAST (GROUP 5)

One gram of baker's yeast per rat was added daily to the diet.

RAT 17.—A female 3 months and 27 days old, weighing 219 Gm., was on this diet for seven weeks without showing any neurologic signs. The diet was completely withdrawn. On the fourth day there developed tonic convulsions. The animal died five days after the beginning of starvation, weighing 156 Gm.

Autopsy.—Grossly, there were no pathologic changes. Microscopically, with the Marchi stain the peripheral nerves disclosed disintegration of the myelin sheaths. The periphery of the spinal cord stained poorly without any alterations being revealed in the myelin sheaths. In the cresyl violet preparation there was vacuolation of the ganglion and anterior horn cells.

RAT 18.—A female 4½ months old, weighing 214 Gm., had been kept on this diet for sixty-three days when it became drowsy. On the seventy-fourth day ataxia, convulsions and opisthotonos set in. After two weeks of this diet there was a continuous loss in weight. The animal was killed on the seventy-fifth day, when it weighed 152 Gm.

Autopsy.—Grossly, there were no changes. Microscopically, with the Marchi stain the peripheral nerves showed very slight disintegration of the myelin sheaths. There were no changes in the ganglion and anterior horn cells.

RAT 19.—A female 4½ months old, weighing 129 Gm., had been on this diet two weeks when a continuous loss in weight developed. The animal was killed on the seventy-second day, when it weighed 98 Gm. There were no neurologic signs.

Autopsy.—Grossly, there were no changes. The peripheral nerves did not disclose disintegration of the myelin with the Marchi or other stains. There was an occasional vacuolated nerve cell in the mesencephalon. The cells of the anterior horns showed slight vacuolation and liquefaction necrosis.

OBSERVATIONS IN CONTROLS (GROUP 6)

Rats were placed on normal stock diet. Some were subsequently starved.

RAT 20.—A male 3 months and 3 weeks old, weighing 188 Gm., was kept on the normal stock diet for two and one-half months, after which it was killed. Neither the peripheral nerves nor the central nervous system showed histologic lesions.

RAT 21.—A male 3 months and 3 weeks old, weighing 234 Gm., was kept on the normal stock diet for a period of two and a half months. The animal was finally starved totally and died six days after the onset of starvation. Neurologic signs and symptoms were absent.

Autopsy.—Grossly, moderate subarachnoid hemorrhages were found over the cerebral and cerebellar surfaces. Microscopically, the peripheral nerves of the fore and hind limbs, stained by the Marchi method (fig. 5), disclosed disintegration of the myelin sheaths. Some of the myelin sheaths of the peripheral nerves stained poorly by the Spielmeyer and sudan III methods. The fiber tracts of

the spinal cord and higher centers were intact. In the cresyl violet preparation the anterior horn and other ganglion cells of the nervous system disclosed vacuolation and liquefaction necrosis.

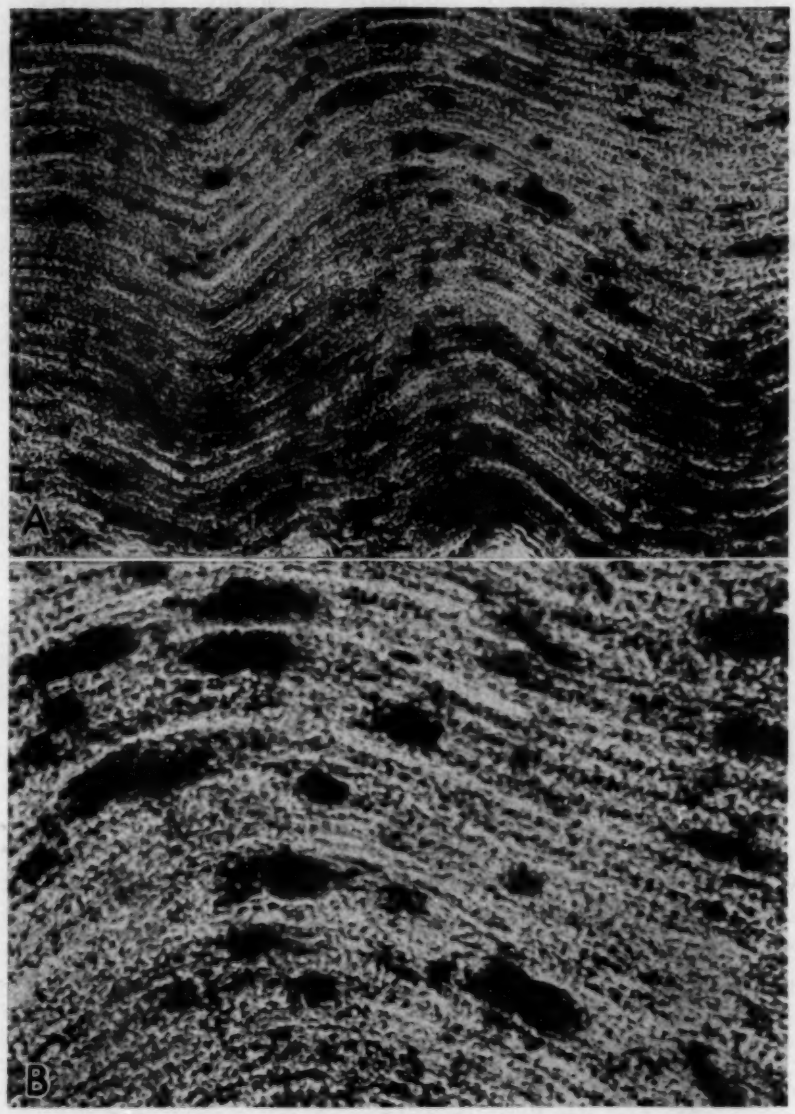

Fig. 5.—*A*, degeneration of a peripheral nerve of a rat subjected to total starvation; Marchi stain; × 240. *B*, same as figure 9; × 480.

Rat 22.—A male 3 months and 3 weeks old, weighing 194 Gm., was kept on the normal stock diet for two and a half months. At the end of this period the

animal was starved for a period of six days, except that he was given 1 Gm. of bemax and 1 Gm. of yeast daily. After five days of starvation, and one day before death, there appeared drowsiness, falling on attempting to walk and priapism.

Autopsy.—Grossly, there were subarachnoid hemorrhages over the cerebral cortex and cerebellum. In the Marchi preparation, the peripheral nerves disclosed moderate breaking down of the myelin sheaths. There were vacuolation and chromatolysis in the anterior horn and other ganglion cells of the nervous system.

COMMENT

Clinical.—The rats that were deprived of vitamin B_1 or of both vitamins B_1 and B_2 showed neurologic signs and symptoms such as weakness, dragging or paralysis of one or more extremities in about thirty to forty days after the administration of the diet and about two to seven days before death. Rat 9 presented these signs fifty-eight days after the initiation of the diet. Some of the animals also exhibited convulsive movements (rats 1, 2, 4, 5, 6, 7, 8, 11, 12 and 18), tonic retractions of the head and hypertonus (rats 2, 3, 8, 9, 11 and 18), ataxia (rats 9, 10, 11, 13 and 18) and priapism (rats 2, 3 and 9). The convulsive movements and the tonic retractions of the head appeared somewhat later than the paralysis of the limbs, generally one to six days before death. Most of the animals died within thirty-five to fifty days after the initiation of the vitamin-free diet, except rat 9, which lived sixty-two days.

The rats that were on a vitamin B_1 deficient diet plus adequate quantities of bemax or yeast were free from neurologic signs, except rat 18. This animal became sleepy and slow in its movements and had equilibratory difficulties after sixty-three days on this diet. One day before it was killed, on the seventy-fourth day, it became ataxic and had opisthotonic convulsions. It lost weight constantly and ate very poorly. We cannot explain this unless we assume that this rat had an especially high vitamin requirement which was not satisfied by his daily ration of yeast, although this was adequate or almost adequate for the other rats. It is noteworthy that the weight curves of the rats in this group do not compare favorably with the others and that slight neuropathologic changes were found in all. The slight equilibratory disturbances noted in rat 14 shortly before death occurred during the final period of starvation, after normal development on a deficient diet plus adequate quantities of bemax.

The three rats which received the stock diet showed no neurologic signs while on this diet. Rat 22 was starved but received yeast and bemax (1 Gm. of each) daily. Five days after the beginning of starvation and one day before death there appeared drowsiness, slight equilibratory disturbances and priapism.

Of the rats which were totally starved, rat 2 of group 1 died without disclosing neurologic signs; rat 20 of group 6 had tonic convulsions the day before death. Of the rats which were starved although provided with abundant vitamin B, rat 22 of group 6 showed drowsiness, locomotor disturbances and priapism the day before death; rat 14 of group 4 showed slight equilibratory disturbances the day it died.

Pathologic.—The changes in the organs other than the nervous system will be omitted in this presentation.

Gross Findings: The brain and spinal cord showed no changes grossly except hemorrhages in the region of the mesencephalon and subarachnoid hemorrhages in some animals deprived of vitamin B_1 and some deprived of B_1 and B_2 and subarachnoid hemorrhages in two of the starved animals.

Microscopic Observations: The animals totally deprived of vitamin B_1 or of vitamins B_1 and B_2, as well as those that received small quantities of bemax or yeast, showed the following lesions.

The peripheral nerves of the extremities disclosed disintegration of the myelin sheaths, best seen in the Marchi and myelin sheath preparations (fig. 1). In the Marchi-stained sections the areas of degeneration were characterized by deposition of black granules, and in the sections prepared by the Spielmeyer method for staining myelin sheaths, by slight areas of demyelinization. Lipoid deposits could not be demonstrated in the sudan III preparations. Slight disintegration of the axis-cylinders was occasionally seen in the Bielschowsky preparations.

The sections of some of the spinal cords when stained by the Marchi method showed at the periphery of the cord an occasional disintegration of a myelin sheath. By the Spielmeyer technic some of the tracts of the spinal cord, especially those of the posterior columns, had a honeycomb appearance (fig. 2). With higher magnification, however, the small defects in the tracts were identified as artefacts due to fixation. By the Marchi or sudan III methods no fatty deposits could be demonstrated within these areas.

The ganglion cells of the mesencephalon, metencephalon and anterior horns disclosed vacuolation, liquefaction necrosis and chromatolysis (fig. 3). Nerve cell changes of the same type were also observed occasionally in the archicortex and neocortex.

In some of the animals with convulsions there were hemorrhages in the mesencephalon and metencephalon with pathologic changes in the glia and ganglion cells (fig. 4). Ischemic or necrobiotic changes such as those described by Spielmeyer in epilepsy or those by Zimmerman and Burack in their dog 4 which was deprived of vitamin B complex were not encountered in the hippocampus or cerebellum of any of our animals.

The animals which were given basic diets deficient in vitamin B_1 or in vitamins B_1 and B_2 but which received adequate quantities of bemax or yeast, excepting the animals subjected to starvation, did not show any significant histologic changes. Very slight changes were present in those animals which were fed 1 Gm. of yeast per rat daily. This ration apparently represented the borderline between adequacy and deficiency.

The animals which received the normal stock diet and were not subjected to starvation did not present any pathologic changes. The rats on the normal stock diet and the rats which received adequate quantities of bemax or yeast which were finally subjected to starvation exhibited the same histologic changes as those which were deprived of vitamin B_1 or of vitamins B_1 and B_2. The peripheral nerves of the totally starved animals showed especially extensive disintegration of myelin (fig. 5). Subarachnoid hemorrhages were also a prominent feature in the rats subjected to total starvation.

REVIEW OF THE LITERATURE

In order to evaluate the clinical and pathologic observations in this experiment, it would be advisable to review briefly the reports of other observers. The first experimenters to induce neural changes by producing nutritional deficiency did not limit themselves to the elimination of the vitamin B complex. Later investigators (Hofmeister,[2] Woollard,[3] Stern and Findlay,[4] Gildea, Kattwinkell and Castle,[1] Marrian, Baker, Drummond and Woollard,[5] Zimmerman and Burack[6] and Grinker and Kandel[7]) eliminated from their diets, as far as possible, only the vitamin B complex.

Hofmeister[2] fed rats on a diet which was deficient only in vitamin B. Clinically, he described neurologic signs, the main symptom complex being cerebellar ataxia. Of fourteen rats, he found peripheral nerve changes in only six, and these were slight or insignificant. On the other hand, in all of the rats severely affected he found small capillary hemorrhages in the cerebrum, brain stem and cerebellum. The extent of the hemorrhages was parallel with the intensity of the clinical neural signs during life. Hofmeister[2] regarded the hemorrhages as cerebral purpura, analogous to the so-called hemorrhagic encephalitis seen in chronic alcoholic, lead or arsenical poisoning. Degenerative chromatolytic nerve

2. Hofmeister, F.: Biochem. Ztschr. **128**:540, 1922.
3. Woollard, H. H.: J. Anat. **61**:283, 1927.
4. Stern, R. O., and Findlay, G. M.: J. Path. & Bact. **32**:63, 1929.
5. Marrian, G. F.; Baker, L. C.; Drummond, J. C., and Woollard, H. H.: Biochem. J. **21**:1336, 1927.
6. Zimmerman, H. M., and Burack, E.: Arch. Path. **13**:207, 1932.
7. Grinker, R. R., and Kandel, E.: Arch. Neurol. & Psychiat. **30**:1287, 1933.

cell changes were also present. He was of the opinion that in man and the dog most of the changes were in the peripheral nerves, while in rats and birds the cerebellum and other higher centers were involved.

Woollard [3] thought that the beriberi occurring in rats might be due to starvation from inability to assimilate food in the absence of appropriate vitamins. Sundwall [8] produced nerve lesions, such as vacuolation and chromatolysis in the ganglion cells and degeneration of fiber tracts in the spinal cord, by starvation and compared these with the neural changes in pellagra. He concluded that pellagra does not possess any characteristic cell alterations and that the pathologic changes result from malnutrition.

Even earlier observers, Chamberlain, Bloombergh and Kilbourne [9] and Eijkman and Von Hoogenhuyze,[10] concluded that inanition in birds produces a condition indistinguishable from beriberi, with a similar pathology of the nervous system.

Stern and Findlay [4] deprived three groups of rats of vitamin B_1, vitamin B_2 and both, respectively. Of these, the group deprived of B_1 showed the highest incidence of neurologic signs; those deprived of B_2, the lowest. Peripheral nerve changes were slight or absent, the principal change being an increase in the II granules of Reich. The anterior horn cells of the spinal cord, however, showed early chromatolysis, shrinkage, abnormal staining and satellitosis. The predominant changes varied with the different groups.

Gildea, Kattwinkell and Castle [1] recorded numerous demyelinized foci in the spinal cords of dogs and thought that they were confronted with lesions which resembled those observed in subacute combined degeneration. Zimmerman and Burack [6] have demonstrated that these demyelinized foci were artefacts in the Spielmeyer technic of staining myelin sheaths. Gildea, Castle, Gildea and Cobb,[11] on repeating their experiments in the dog, insisted that the areas of demyelinization in the spinal cord are not artefacts produced by the Spielmeyer technic, since the same changes were observed in the sections stained by the Pal-Weigert method. In their last experiment the authors described areas of demyelinization in the spinal cords of seven dogs and myelin degeneration of the peripheral nerves in three dogs. Gildea, Castle, Gildea and Cobb [11] criticized the minor lesions of the nervous system described by Woollard and Drummond in inanition and cachexia on the basis that the

8. Sundwall, J., and Francis, E.: Hygienic Laboratory Bulletin 106, 1917, p. 5.

9. Chamberlain, W. P.; Bloombergh, H. D., and Kilbourne, E. D.: Philippine J. Sc. **6**:177, 1911.

10. Eijkman, C., and Von Hoogenhuyze, C. J. C.: Versl. k. Akad. v. Wetensch. te Amsterdam **18**:1467, 1916.

11. Gildea, M. C. L.; Castle, W. B.; Gildea, E. F., and Cobb, S.: Am. J. Path. **11**:669, 1935.

animals may have been deprived of vitamin A at the same time that they were starved.

Woollard,[3] and later Marrian, Baker, Drummond and Woollard,[5] described nervous symptoms similar to those observed in pigeons suffering from vitamin B deficiency in a number of starving birds. They also concluded that the convulsions observed could not be due to vitamin B deficiency since several cases were observed in birds receiving daily rations of vitamin B.

Zimmerman and Burack[6] found extensive demyelinization in the peripheral and vagus nerves of dogs deprived of water-soluble B vitamins. They also noted ganglion cell changes and disseminated foci of myelin destruction in the brain and spinal cord but believed that these could not be held responsible for the clinical symptoms of the nutritional disorder. Dogs deprived wholly or in part of the thermostabile B_2 factor showed large zones of degeneration and vascular proliferation in the vermis of the cerebellum and in the region of the substantia gelatinosa Rolandi. Peripheral neuritis of a lesser degree was also present in control animals that were entirely deprived of food but supplied with an adequate amount of B complex.

Grinker and Kandel[7] were unable to demonstrate lesions of the nervous system in rats deprived of the vitamin B complex. Gildea and co-workers maintained that Grinker and Kandel had failed to find evidence of lesions of the nervous system in rats deprived of the vitamin B complex probably "due either to the fact that the animals died in acute illness, or to unsuitability of the species for this study."

SUMMARY

In our experiments, vacuolation, liquefaction necrosis and chromatolysis of ganglion cells in the mesencephalon, metencephalon and spinal cord, as well as disintegration of the myelin sheaths of the peripheral nerves, were found alike in the animals subjected to diets totally and subtotally deficient in vitamin B_1 or in both vitamins B_1 and B_2 and in those suffering from pure inanition. Neurologic symptoms such as ataxia, dragging of the extremities, convulsions, priapism and tonic retractions of the head occurred in the rats that died of nutritional deficiency. They were less marked in the controls subjected to pure inanition than in the animals deprived of vitamin B_1 or of vitamins B_1 and B_2, probably because of the short duration of life in the former group.

The question of the relationship between the neural symptoms of the rats and the histologic changes described cannot be easily answered. The paresis or paralysis of the extremities may be ascribed to the peripheral neuritis. The convulsions and the tonic retractions of the head may be attributed to the hemorrhages and nerve cell changes in the

mesencephalon and metencephalon. These findings were analogous to those described by Hofmeister, who fed rats on a diet which was deficient only in vitamin B. The ataxia, asthenia and equilibratory disturbances may be due to inanition and weakness. It should be emphasized that the deprivation of vitamins expresses itself very early in loss of appetite with consequent inanition.

The specific ganglion cell changes, of which vacuolation was the most prominent, were of the type of those encountered in nutritional diseases, high fever, extreme exhaustion, prolonged diseases, drug intoxications and technical defects due to fixation. In view of the uniformity with which these changes occurred and of the special care taken in the fixation and staining of the tissues of these experimental animals we consider that the changes represent a true pathologic state. The histologic changes as well as the clinical phenomena in the inanition group were less severe than in the other groups, except in the peripheral nerves, where the changes were more marked. The administration of abundant vitamin B to the animals subjected to starvation did not prevent the occurrence of pathologic changes essentially similar to those occurring in the animals deprived of vitamin B_1 or of vitamin B_1 and B_2. This seems to indicate that inanition is an important factor in the direct pathogenesis of the lesions associated with a deficiency of vitamin B. The anorexia resulting in inanition is most likely caused by the avitaminosis.

CONCLUSIONS

Diets deficient in vitamin B_1 or in vitamins B_1 and B_2 (totally and subtotally deficient) result in the rat in neurologic signs such as dragging and paralysis of the extremities, equilibratory disturbances, priapism, convulsions and tonic retractions of the head.

Pathologically, the outstanding picture is that of disintegration of the myelin sheaths of the peripheral nerves and vacuolation and liquefaction necrosis of the ganglion cells of the mesencephalon, metencephalon and anterior horns of the spinal cord. Hemorrhages with changes in the ganglion and glia cells of the mesencephalon are also encountered in the animals with convulsions.

The pathologic changes in the nervous system in animals suffering from inanition are essentially the same as those in animals deprived of vitamin B_1 or of both vitamins B_1 and B_2. If anything, the changes in the peripheral nerves in animals subjected to inanition are more marked. In such animals the pathologic changes are essentially the same whether the animals are totally starved or are given abundant vitamins while starving. The clinical manifestations in animals subjected to inanition, however, are not identical with those in animals suffering from vitamin B deficiency, possibly because of the rapid death of the starved animals.

SPECIFIC HISTOLOGY OF GRANULOMA INGUINALE

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The variance in histologic descriptions of granuloma inguinale is so gross as to find its parallel in the diversity of opinions as to the nature of the causal organism, that is, the Donovan body.[1] Incongruous as it may seem, many investigators [2] have described the lesion histologically but have failed to recognize certain evident and definitive facts. Our purpose in this paper is to chronicle certain observations that provide a specific picture which permits differentiation of granuloma inguinale from other granulomatous lesions.

The clinical diagnosis of a lesion on or about the genitalia is often difficult and may necessitate biopsy. The correct diagnosis is important because frequently specific remedies are indicated. The chancre and condyloma of syphilis, the chancroid, the inguinale granuloma and the venereal lymphogranuloma, as well as carcinoma, are encountered on the pudenda often as mixed infections. Particularly among Negroes is it a frequent observation that one patient may be afflicted with two or more of the diseases in some combination or permutation. This possibility has probably been insufficiently considered in previous histologic descriptions of granuloma inguinale.

Biopsy of tissues presenting these various lesions often results in descriptive rather than diagnostic reports by pathologists, because the nature of the lesions is not readily recognized. In sections from a chancre and a syphilitic condyloma special stains usually reveal Spirochaeta pallida. To this specific diagnostic picture we wish to add another that makes the diagnosis of granuloma inguinale possible from histologic study.

A group study of the various entities that comprise the venereal diseases is now being pursued in the medical school of the University of

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From the Department of Pathology, University of Georgia School of Medicine.

1. Walker, E. L.: J. M. Research **37**:427, 1918. Johns, F. M., and Gage, I. M.: Internat. Clin. **4**:15, 1924. McIntosh, J. A.: J. A. M. A. **87**:996, 1926.

2. Symmers, Douglas, and Frost, A. D.: J. A. M. A. **74**:1304, 1920. Campbell, M. F.: ibid. **76**:648, 1921. Lynch, K. M.: ibid. **77**:925, 1921. Goodman, H: ibid. **79**:815, 1922. Fox, H.: ibid. **87**:1785, 1926. Grindon, Joseph: Arch. Dermat. & Syph. **31**:236, 1913. Weinberg, M.: J. Urol. **9**:505, 1923.

Georgia.[3] This study has enabled us to recognize a histologic picture specific for granuloma inguinale, and we deem it worth while at this time to publish a short report.

Seven recent cases of granuloma inguinale, in which the diagnosis was made by the demonstration of Donovan bodies in stained films, received careful clinical study. Sections were removed from the involved tissues, and a histologic study was undertaken. The tissues were fixed in neutral 10 per cent formaldehyde solution, embedded in paraffin, sectioned, and stained by Delafield's hematoxylin method and with eosin. Sections stained by this method were found preferable to sections stained with graded Giemsa stain[4] from p_H 5.2 to 5.6 or tissue fixed in Bouin's solution.[5] In the sections there were encountered, in variable numbers, large cells of a peculiar type that contained many intracytoplasmic spaces which in turn contained small deeply stained bodies. Moreover, numerous sections of chancroids, chancres, condylomas and venereal lymphogranulomas, which served as controls, did not exhibit these peculiar cells. A definite pathologic picture was also noted in sections of granuloma inguinale. A search through the files in the department revealed that seven other cases had been observed there which exhibited the pathognomonic cell as well as the characteristic histologic picture. The sections in these cases had been diagnosed merely as "granulomatous tissue." Moreover, the clinical histories of these cases indicated the probability that granuloma inguinale was present. The presence of the pathognomonic cell in the sections, we feel, definitely places the cases in this category.

THE PATHOGNOMONIC CELL

The pathognomonic cell is a large mononuclear cell that varies from 25 to 90 microns in diameter. The origin of the cell is not clear, and we are not prepared to say whether it is a histiocyte derived from the fixed tissues, an altered plasma cell or an endothelial leukocyte. The intracytoplasmic cysts filled with deeply stained bodies are the outstanding feature. Some cells have but a single cyst, while the majority

3. Greenblatt, R. B.; Sydenstricker, V. P., and Pund, E. R.: J. M. A. Georgia **26**:16, 1937. Greenblatt, R. B., and Wright, J. C.: Am. J. Syph., Gonor. & Ven. Dis. **20**:654, 1936. Greenblatt, R. B., and Sanderson, E. S.: Chancroidal Vaccine: A Method of Preparation; Its Diagnostic and Therapeutic Value, Am. J. Clin. Path., to be published. Dienst, R. B., and Sanderson, E. S.: Am. J. Pub. Health **26**:910, 1936. Sanderson, E. S., and Greenblatt, R. B.: South. M. J. **30**:147, 1937.

4. Cornwall, L. H., and Peck, S. M.: Arch. Dermat. & Syph. **12**:613, 1925. Randall, A.; Small, J. C., and Belk, W P.: Surg., Gynec. & Obst. **34**:717, 1922.

5. Bouin's solution contains saturated aqueous solution of trinitrophenol, 75 parts; dilute solution of formaldehyde U. S. P., 25 parts; glacial acetic acid, 5 parts.

of them have as many as from ten to twenty. The deeply stained bodies within these cysts are grouped peripherally. However, by altering the focus of the lens other encysted bodies as well as numerous cysts are demonstrated at different levels. These bodies appear round, occasionally rodlike, and have an affinity for hematoxylin. The nucleus of the cell, eccentrically placed, is usually pyknotic. As the intracytoplasmic cysts

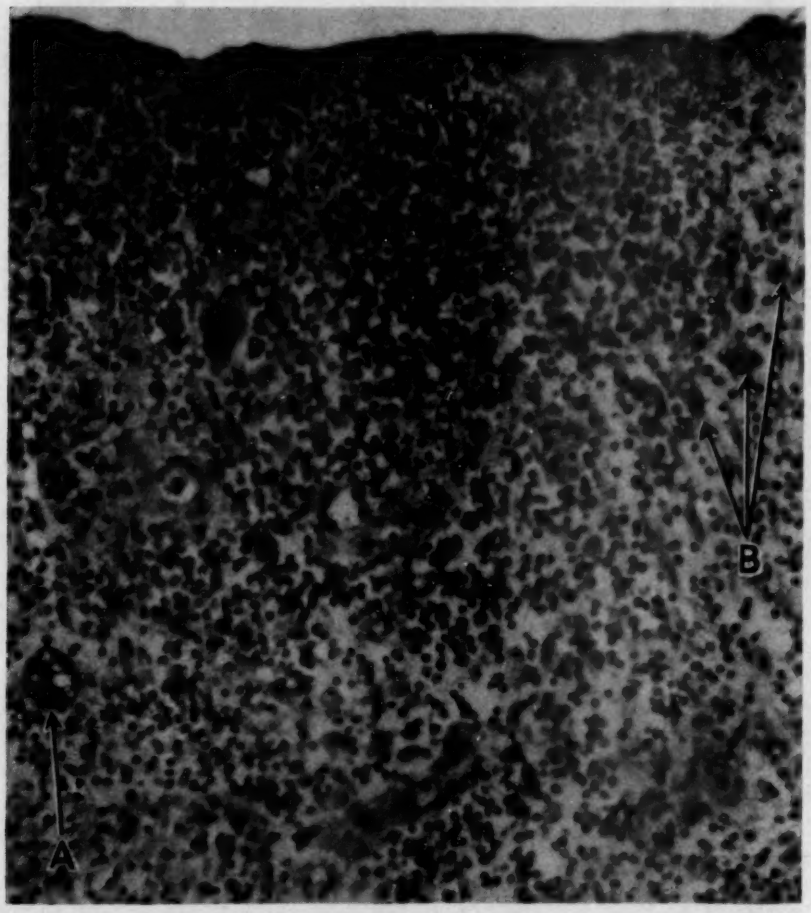

Fig. 1.—The characteristic dense cellular response in which the pathognomonic cells at *A* and *B* are diagnostic, thus permitting the diagnosis of granuloma inguinale.

multiply and enlarge the nucleus is further crowded to one side and often flattened against the cell membrane. Frequently the cell itself becomes a multiloculated cyst in which the cytoplasm and nucleus have been completely effaced. The cytotropism of the bodies within the cysts is signally demonstrated in histologic study; their tropism for

these particular cells and their intracellular disposition within the cytoplasmic cysts identify them with the Donovan bodies. The presence of multiple cysts is interpreted to mean intracellular multiplication.[6]

The cell described, we feel, is as significant as the Sternberg-Reed cell is in Hodgkin's disease and as diagnostic as the tubercle bacillus and Spirochaeta pallida are in tuberculosis and syphilis.

THE HISTOLOGY

Histologically, granuloma inguinale is characterized by a luxuriance of granulation tissue which is surcharged with plasma cells. The massiveness of the cellular response is one of the features of this granulation tissue, and mitotic figures are frequently demonstrable. Conspicuously few are the lymphocytes. With or without ulceration there are seen a variable number of polymorphonuclear leukocytes, mainly neutrophilic, but occasionally eosinophils, too, are observed. The leukocytes are found in larger numbers near the surface. In very active lesions focal miliary abscesses are noted in the superficies of the ulcer and in the papillae at the margins. This process involves primarily the corium but extends into the subjacent subcutaneous tissue.

The floor of the ulcer is composed of granulation tissue, which sometimes becomes exuberant. The marginal epithelium usually shows pronounced proliferative changes. The epithelial hyperplasia may be so great and the granulation tissue reaction in the papillae so massive that the rete pegs become elongated and the resultant relationship so distorted that islands of keratinized epidermis are frequently found deep in the section. These give the appearance of epithelial pearl formation and may simulate early carcinoma.

As the granulomatous tissue in the papillae becomes pronounced, the overlying hyperplastic epithelium finally becomes attenuated. As focal papillary abscesses develop, necrosis of the overlying epithelium follows, and in this manner there is slow progression of the ulceration.

At nearby points, from 2 to 4 cm. distant from the primary lesion, secondary zones of infection appear. These manifest themselves as well defined areas of papillary excrescences, as evidence of the intense epithelial stimulation. These secondary foci, it appears, result not from inoculation but from centrifugal spread of the infection along the corium and subcutaneous tissue.

The pathognomonic cells are scattered in variable numbers in the superficial half of the granulation tissue and in the papillary layer of the corium, particularly near the margins of the ulcer. These cells are found sometimes only after prolonged search with the oil immersion objective, but in the exuberant granulation tissue they are usually so abundant as to be readily recognized with low power magnification.

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6. De Monbreun, W. A., and Goodpasture, E. W.: Am. J. Trop. Med. **9**:311, 1931.

Certain pitfalls are to be avoided in the recognition of the pathognomonic cell. In various types of granulomatous lesions three types of cells may offer difficulties in differentiation: 1. In a degenerating endothelial leukocyte the nucleus frequently becomes large and clear, with peripheral distribution of the chromatin. 2. Hydropic cells show

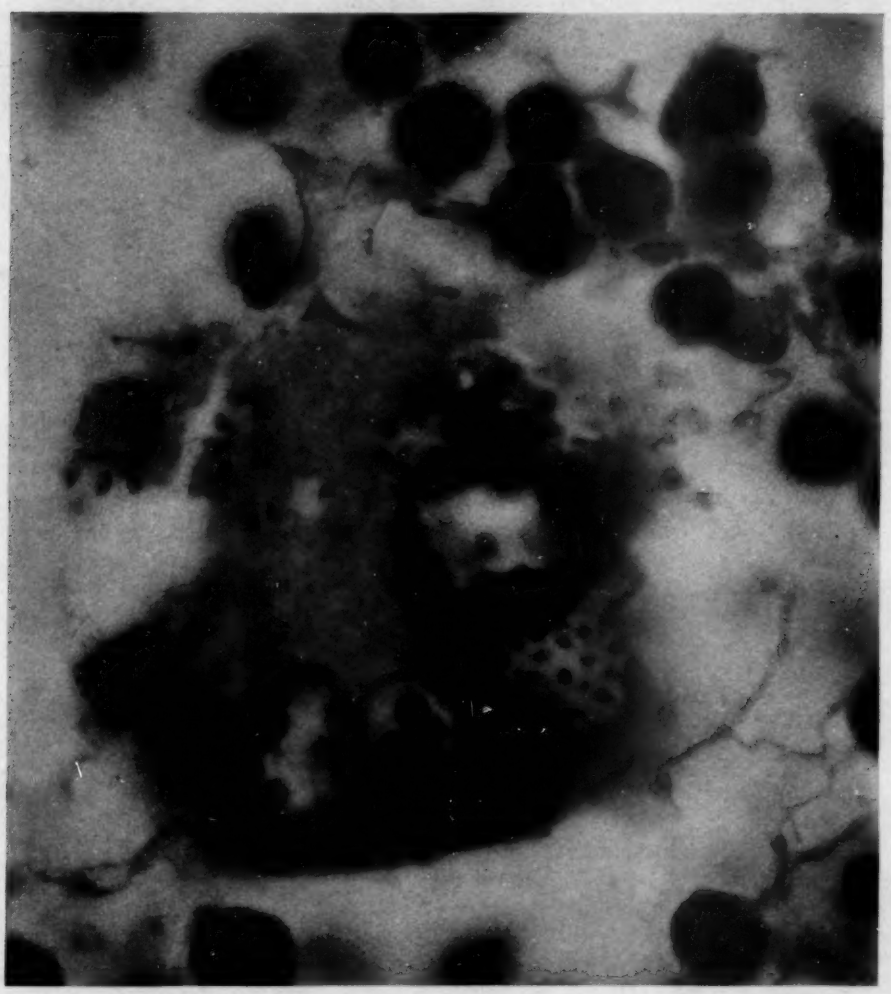

Fig. 2.—The pathognomonic cell. Note the small rounded bodies within the intracytoplasmic cysts. Compare this cell in size with the neighboring polymorphonuclear leukocytes and plasma cells.

single or multiple vacuoles but are readily ruled out by the absence of the characteristic bodies within the vacuoles. 3. Mast cells contain coarse granules but are differentiated by the absence of cysts.

SUMMARY

Granuloma inguinale reveals in the pure or unmixed cases a uniform histologic picture. The essential features are (*a*) the massiveness of the cellular reaction in which the luxuriant granulation tissue is surcharged with plasma cells, (*b*) the relative and conspicuous paucity of lymphocytes, (*c*) the diffuse sprinkling of polymorphonuclear leukocytes, with focal collections in the superficies and papillae, (*d*) the pronounced marginal epithelial proliferation simulating early epitheliomatous changes and (*e*) the pathognomonic large mononuclear cells scattered in various numbers throughout the granulation tissue.

The pathognomonic cell is specific for granuloma inguinale. The relatively large size of the cell, the diameter of which varies from 25 to 90 microns, and the many intracytoplasmic cysts filled with deeply stained bodies are its cardinal features. These bodies are round or rod-like, are grouped peripherally within the cysts and have an affinity for hematoxylin. The recognition of this cell is of paramount importance, for it permits the diagnosis of granuloma inguinale to be made by histologic study of the tissue.

RÔLE OF ANAEROBIC STREPTOCOCCI IN HUMAN INFECTIONS

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Anaerobic streptococci, while rare as pathogens, are assuming a position of increasing prominence as the etiologic factor in certain inflammatory conditions. Hitherto, anaerobic streptococci have been recovered mainly in cases of puerperal and pulmonary sepsis, whereas the lesions to be described vary somewhat in situation from those reported in the literature. The object of this investigation was to determine the type of lesion encountered in such infections, as well as the biologic and agglutinative properties of the organisms recovered, and to study the pathogenicity of anaerobic streptococci for animals.

Veillon [1] was the first to describe anaerobic streptococci. He recovered a strictly anaerobic coccus, which he called "Micrococcus foetidus," from three sources, namely, Ludwig's angina, perinephric abscess and bartholinitis (two cases). In only one case, namely, one of the cases of suppurative bartholinitis, were the anaerobic streptococci grown in pure culture. Both the pus and the cultures were foul smelling. The organisms grew in short chains and were larger than ordinary pyogenic streptococci. Injected in large amounts into animals, the culture material produced small subcutaneous abscesses. Krönig [2] and Menge [3] independently reported the recovery of anaerobic streptococci from the vagina of the normal pregnant woman. It was noted that one group of streptococci produced gas and foul odor in cultures. Later, Krönig and Menge [4] cultured several similar strains from the lochia of women who had puerperal septicemia. Natvig [5] recovered six strains of anaerobic streptococci from infected lochia, all of which, like Krönig and Menge's strain, produced gas in culture mediums. He suggested that

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1. Veillon, M. A.: Compt. rend. Soc. de biol. **45**:807-809, 1893.
2. Krönig: Zentralbl. f. Gynäk. **19**:409-412, 1895.
3. Menge: Zentralbl. f. Gynäk. **19**:433-434, 1895.
4. Krönig and Menge, quoted by Taylor.[17]
5. Natvig, Harald: Arch. f. Gynäk. **76**:700-859, 1905.

the entire group of anaerobic streptococci should be called "Streptococcus anerobius" (Krönig). He failed to produce lesions in animals. A different strain of anaerobic streptococcus was isolated from the mouths of normal suckling infants (Lewkowicz [6]). This organism was very minute (diameter, from 2.5 to 4 microns) and did not produce gas on culture mediums. It was named "Streptococcus anerobius micros." Schottmüller [7] described anaerobic streptococci which he had recovered from the blood in cases of puerperal sepsis but which were different from the strain described by Krönig. These streptococci failed to produce gas in cultures except in the presence of blood. He called this organism "Streptococcus putridus" because he thought it was responsible for the putrid condition of the blood. It produced no lesions in animals. In reviewing the etiology of puerperal sepsis, Schottmüller [8] expressed the belief that anaerobic streptococci were responsible for two thirds of the deaths from this condition. Schwarz and Dieckmann [9] and Colebrook [10] obtained similar results in their studies of puerperal infections.

Anaerobic streptococci were isolated from a number of war wounds. Cottet [11] isolated ten strains from thirty-three gunshot wounds. Gérard and Romant [12] recovered a strictly anaerobic streptococcus ten times from 117 wounds. In a bacteriologic study of 192 fallopian tubes which had been removed surgically Curtis [13] found five strains of anaerobic streptococci but did not classify them. The best work on classification of the anaerobic streptococci was done by Prévot,[14] who reported thirteen strains of strictly anaerobic streptococci. The sources from which he isolated the organisms included pulmonary gangrene, puerperal sepsis and appendical abscesses. He added one strain to those already described; this strain he called "Streptococcus intermedius." He reported that this organism was a medium sized diplococcus (diameter, 0.6 micron) which produced neither gas nor odor in cultures but coagulated milk. Suspensions of live organisms injected alone into guinea-pigs and mice produced only small abscesses, but injected with a filtered

6. Lewkowicz, Xavier: Arch. de méd. expér. et d'anat. path. **13**:633-660, 1901.

7. Schottmüller, Hugo: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **21**:450-490, 1910.

8. Schottmüller, Hugo: München. med. Wchnschr. **75**:1580-1583 and 1634-1638, 1928.

9. Schwarz, O. H., and Dieckmann, W. J.: Am. J. Obst. & Gynec. **13**:467-485, 1927.

10. Colebrook, Leonard: Brit. M. J. **2**:134-137, 1930.

11. Cottet, J.: Compt. rend. Soc. de biol. **81**:6-9, 1918.

12. Gérard, P., and Romant: Compt. rend. Soc. de biol. **82**:136-138, 1919.

13. Curtis, A. H.: Surg., Gynec. & Obst. **33**:621-631, 1921.

14. Prévot, A. R.: Ann. Inst. Pasteur **39**:417-447, 1925.

toxin of Vibrion septique they produced widespread crepitant lesions. High titers of homologous agglutinins were obtained with some strains, particularly with Streptococcus anaerobius (1:25,000), and some cross-agglutination was obtained between several strains. Harris and Brown [15] isolated seventeen strains of anaerobic streptococci from the vaginas of thirty normal women in the puerperium. Harris and Brown [16] studied fifty-seven strains of anaerobic streptococci from infected lochia of one hundred and sixty-eight women in the puerperium. Forty-nine strains produced no hemolysis or coloration on blood agar, and twenty-three strains failed to ferment any of the eight sugars tried. Only one strain fermented mannite. Practically no pathogenicity for animals was found. Harris and Brown suggested a classification based on the reactions to blood and sugars. Taylor [17] reported on eleven strains of strictly anaerobic streptococci obtained from infections of frontal sinuses, pleurisy, pulmonary gangrene and dental sepsis. Because of the variations observed in the fermentation of sugars, he concluded that such a method of classification would be useless. Soule and Brown [18] obtained anaerobic streptococci from cervical cultures in 40 per cent of the women examined.

MATERIAL

Our study includes the cases occurring between 1931 and 1936 in which the patients came to necropsy and in which anaerobic streptococci were recovered either from the blood or from the lesions at the time of death. Further bacteriologic work was done on the nine strains isolated in 1935 and 1936, as well as on two strains obtained in cases in which the patients survived.

PATHOLOGIC CHANGES

In the twenty-three cases in which anaerobic streptococci were recovered at necropsy (table 1) some part of the intestinal tract was involved nine times, the lung primarily seven times (figure) and the meninges four times; in three cases the lesions occurred in various tissues. Abscess of the lung was the most predominant pulmonary lesion in four cases. Gangrenous appendicitis with rupture accounted for five of the intestinal lesions. These were usually followed by formation of abscesses; in one case there was not time for an abscess

15. Harris, J. W., and Brown, J. H.: Bull. Johns Hopkins Hosp. **43**:190-200, 1928.

16. Harris, J. W., and Brown, J. H.: Bull. Johns Hopkins Hosp. **44**:1-31, 1929.

17. Taylor, A. L.: The Anaerobic Streptococci, in Bensted, H. J., and others: System of Bacteriology, Medical Research Council, London, His Majesty's Stationery Office, 1929, vol. 2, pp. 136-142.

18. Soule, S. D., and Brown, T. K.: Am. J. Obst. & Gynec. **23**:532-537, 1932.

to develop. There were three cases in which carcinoma of the large bowel had perforated and produced an abscess. In one case a duodenal ulcer had perforated two and a half months previously and had been surgically closed. At necropsy a huge subdiaphragmatic abscess was found, with empyema on the right side and multiple chronic abscesses of the liver. The duodenal ulcer had entirely healed, and its site was marked merely by a scar.

A longitudinal section of the lung and liver in case 2, showing the empyema and subdiaphragmatic abscess; the exudate is very thick.

The four cases in which anaerobic streptococci were isolated from the meninges represented a type of infection about which practically nothing has been written. The foci in all four cases were different. They represented chronic infections in the nasopharynx, frontal sinus and middle ear. In the fourth case an infected wound in the region of the tenth thoracic vertebra had resulted in the infection.

The duration of the major symptoms varied from four days up to one and a half years. In the majority of cases this could be taken as

TABLE 1.—*Pathologic Changes in Cases in Which Anaerobic Streptococci Were Isolated*

Case	Culture		Primary Lesion	Secondary Lesion	Duration of Symptoms	Histology
	Source	Organisms				
1	Abscess of lung	Anaerobic streptococci, Fusiformis dentium, Borrelia Vincenti and Staphylococcus	Abscess of lung	Pyopneumothorax, bronchopneumonia	3 weeks	Subacute inflammation, beginning organization
2	Subdiaphragmatic abscess	Anaerobic streptococci (pure culture)	Abscess of lung	Subdiaphragmatic abscess, bronchial fistula, bronchopneumonia	2 months	Chronic inflammation, necrosis
3	Abscess of lung	Anaerobic streptococci* and other anaerobes	Abscess of lung	Bronchopneumonia	1½ years	Chronic inflammation, marked fibrosis
4	Abscess of lung	Anaerobic streptococci and Bacteroides funduliformis	Carcinoma of pancreas	Multiple abscesses of lungs with ulceration of bronchi	5 months	Chronic inflammation, changes around abscess
5	Empyema	Anaerobic streptococci, Fusiformis dentium and Borrelia Vincenti	Bronchopneumonia	Empyema	2½ months	Empyema, thick exudate with organization
6	Cardiac blood	Anaerobic streptococci and diphtheroids	Foreign body in bronchus	Tracheobronchitis, bronchopneumonia	5 days	Fibrin and polymorphonuclear leukocytes in lung
7	Phlegmon	Anaerobic streptococci, Fusiformis dentium, Borrelia Vincenti and Staphylococcus aureus	Phlegmon of neck	Bilateral bronchopneumonia with gangrene	14 days	Acute cellular reaction with extensive necrosis
8	Meninges	Anaerobic streptococci† (pure culture)	Hemangioma of nasopharynx	Acute cerebrospinal meningitis	13 days	Acute meningitis
9	Meninges	Anaerobic streptococci† (pure culture)	Otitis media	Thrombosis of the cavernous sinus and acute cerebrospinal meningitis	4 days	Acute meningitis
10	Meninges	Anaerobic streptococci† (pure culture)	Frontal sinusitis	Acute cerebrospinal meningitis	3 weeks	Acute meningitis
11	Meninges	Anaerobic streptococci (pure culture)	Infected wound in region of thoracic segment of spinal cord	Acute cerebrospinal meningitis	34 days	Predominance of lymphocytes in exudate

12	Peritoneum	Anaerobic streptococci, Streptococcus viridans and Escherichia coli	Gangrenous appendicitis	Pelvic abscess, general peritonitis	10 days	Wall of abscess showed subacute inflammation
13	Peritoneum	Anaerobic streptococci, Streptococcus viridans and Streptococcus faecalis	Gangrenous appendicitis with rupture	General peritonitis	5 days	Acute peritonitis, colonies of bacteria in the sections
14	Peritoneum	Anaerobic streptococci, Bacteroides fragilis and so forth	Gangrenous appendicitis with rupture	General peritonitis, beginning abscess formation	17 days	Acute and subacute inflammation
15	Blood of heart	Anaerobic streptococci* (pure culture)	Acute appendicitis with rupture	Pelvic abscess, pseudomembranous jejunitis	1 month	Chronic and subacute inflammation
16	Pelvic abscess	Anaerobic streptococci (predominant) and other anaerobes	Gangrenous appendicitis with rupture	Pelvic abscess, intestinal obstruction	6 months	Chronic inflammation and marked fibrosis in wall of abscess
17	Blood of heart	Anaerobic streptococci and diphtheroids	Carcinoma of hepatic flexure (perforated)	Retroperitoneal abscess perforating into duodenum	6 months	Chronic inflammation
18	Blood of heart	Anaerobic streptococci (pure culture)	Carcinoma of rectum (perforated)	Perirectal abscess, pelvic peritonitis, retroperitoneal and scrotal cellulitis	4 months	Abscess with chronic inflammation, acute peritonitis
19	Blood of heart	Anaerobic streptococci and Escherichia coli	Carcinoma of rectum	General peritonitis	5 days	Acute peritonitis
20	Abscess of liver	Anaerobic streptococci (pure culture)	Chronic duodenal ulcer (perforated)	Abscesses of liver, subdiaphragmatic abscess, empyema	2½ months	Chronic inflammation
21	Umbilical vein	Anaerobic streptococci (pure culture)	Omphalitis	Thrombophlebitis of portal vein, general peritonitis	3 days	Acute phlegmonous inflammation
22	Blood of heart	Anaerobic streptococci (pure culture)	Periurethral abscess	Cellulitis of pelvic fascia, general peritonitis	6 days	Acute inflammation of pelvic tissues, marked necrosis, many cocci
23	Heart	Anaerobic streptococci (pure culture)	Pelvic cellulitis	Pelvic peritonitis	14 days	Subacute inflammation

* After prolonged (from two to three months) cultivation, the organisms became aerobic.
† Able to grow in the depths of blood agar plates after cultivation for from two to three months.

an index of the length of the terminal infection. In only four cases did the terminal illness last less than one week. This chronicity seems to be attributable to the low virulence of anaerobic streptococci.

Microscopically, most of the lesions presented the appearance of a nonspecific chronic granuloma. The exceptions were those which terminated rapidly. In cases 7 and 22, extensive necrosis was the most significant feature. In case 13, there were numerous colonies of gram-positive cocci in the sections of the lesions.

Anaerobic streptococci were recovered in pure culture in eleven of the twenty-three cases. Often they were isolated both from the blood and from the lesions. The frequency with which they were found in pure culture lends importance to these organisms as a cause of human inflammatory disease and suggests that they played a significant rôle in all the cases in which they were present whether or not they were the sole pathogens present. They were mixed with aerobes in seven cases and with anaerobes in seven cases. In two cases the anaerobic streptococci were mixed with both aerobes and anaerobes. From three pulmonary abscesses anaerobic streptococci were recovered in association with Vincent's organisms. Bacilli of the genus Bacteroides were isolated with anaerobic streptococci in two cases.

BACTERIOLOGY

Methods.—The anaerobic plating method described by Spray [19] was used to isolate the colonies from mixed cultures as well as to determine the action of the organisms on blood agar. Strains were grown in tubes (20 by 1.5 cm.) of dextrose brain broth. Failure to grow on or in the depths of aerobic blood agar plates made with beef infusion agar containing 0.25 per cent solution of dextrose was the criterion taken to indicate that the organisms isolated were obligatory anaerobes. Soft agar to which fresh brain had been added was used to keep cultures alive for several weeks.

Fermentation of sugars was determined in tubes of plain brain broth to which had been added 0.5 per cent of the desired sugar, which had previously been sterilized by passing it through a Seitz filter. It was found that growth in the ordinary fermentation tubes without the brain broth was inadequate even after placing the tubes in an anaerobic jar. The marble chips which were usually added to the brain broth to act as a buffer were omitted. After inoculation the tubes were sealed with paraffin and placed in the incubator for three weeks. They were examined frequently to determine if any gas had been produced. At the end of the period of incubation a stained smear was made from the bottom of each tube of culture medium to determine growth and possible contaminants. The p_H of each culture was determined by adding a few drops of bromthymol blue and comparing with color standards. The production of indol was determined by the Ehrlich test. Milk cultures were made in the same long narrow tubes as were used for brain broth; bromcresol purple was used as an indicator.

Considerable difficulty was experienced in preparing satisfactory antigens for agglutination reactions. Growths on slants in anaerobic jars were so uncertain

19. Spray, R. S.: J. Lab. & Clin. Med. **16**:203-206, 1930.

as to be of no value. For this reason, four day old cultures in dextrose brain broth were used for the preparation of the suspensions. The top portion of the brain broth was centrifugated, and the organisms were killed by adding solution of formaldehyde U. S. P. diluted 1 : 500 in physiologic solution of sodium chloride. The organisms were suspended and the chains broken up by shaking vigorously. It was necessary to shake several strains with glass beads for an hour to break up the chains of streptococci. Because of the spontaneous settling of the antigens it was difficult to read the agglutination reactions after leaving the tubes in the incubator at 37 C. for eighteen hours. Satisfactory results could be obtained by placing the tubes in a water bath at 56 C. for two hours, recording the results and checking them after the tubes had remained in the incubator for eighteen hours.

Immune serum was obtained from rabbits each of which had received ten injections of 1 cc. amounts of a suspension which had been diluted to compare with a standard typhoid vaccine containing 500,000,000 organisms per cubic centimeter. Each animal was bled one week after the final injection.

An attempt was made to determine the pathogenicity of several strains for animals as soon as possible after isolation of the strains from the lesions. In one case the pus from a pelvic abscess was used for inoculation. In others, one or two day old brain broth cultures were injected. Rabbits, mice, chickens and guinea-pigs received injections subcutaneously, intramuscularly, intraperitoneally, intravenously and intraspinally. Injections were also made into the testes and into the eyes of rabbits.

Morphology.—All strains appeared to be morphologically similar with a few exceptions. In general, they were medium-sized (0.6 micron) grampositive cocci, which grew mostly in chains of from four to eight and occasionally in longer ones. Strain 22 showed larger cocci (1 micron) with shorter chains and would be classified as Micrococcus foetidus. Strain 10 was constituted of very pleomorphic streptococci, which often appeared as diphtheroids in older cultures.

Biologic Reactions.—The biologic reactions of the various strains of anaerobic streptococci are shown in table 2. Strains 8, 9 and 10, while strictly anaerobic for from two to three months after isolation, would grow at the end of that time in the depths of aerobic blood agar plates made with beef infusion agar containing 0.25 per cent dextrose. They would not grow on blood agar plates made with a plain agar base. It seems possible, therefore, that after prolonged cultivation on artificial mediums some of the strains of anaerobic streptococci become microaerophil in the presence of suitable mediums.

Of the eleven strains studied biologically, only three produced similar reactions on sugar. These three strains were isolated from cerebrospinal meningitis. Another striking feature was that many of the strains fermented very few sugars. It must be mentioned that although growth was present in all the fermentation tubes, in several it was not vigorous and the carbohydrates may not have been attacked. The addition of small amounts of potato or whole blood did not aid growth appreciably. The presence of carbon dioxide in the environment produced no noticeable increase in growth. This finding agrees with the observations of

Harris and Brown.[16] Only one strain produced hemolysis on blood agar, and none was of the viridans type.

Agglutination.—The agglutinative reactions are summarized in table 3. The variations recorded in the titer of serum necessary for complete homologous agglutination can be referred chiefly to the differences in

TABLE 2.—*Biologic Reactions of Anaerobic Streptococci*

	Strains 5 and 16	Strains 8, 9 and 11	Strain 10	Strain 19	Strain 22	Strain 23	Strain 24*	Strain 25*
Dextrose	0	+†	0	0	+	+	+	+
Maltose	0	+	0	+	+	0§	+	+
Sucrose	0	+	0	0	+	+	0	+
Lactose	0	+	0	0	+	+	0	0
Mannite	0	0	0	0	+	0	0	0
Raffinose	0	0‡	0	0	0	+	0	0
Salicin	0	+	0	0	+	0	0	+
Inulin	0	0	0	0	0	0	0	0
Arabinose	0	0	0	0	0	0	0	0
Xylose	0	0	0	0	0	0	0	0
Levulose	0	+	0	+	+	0	0	+
Dextrin	0	+	0	0	+	+	0	0
Dulcitol	0	0	0	0	0	0	0	0
Sorbitol	0	0	0	0	+	0	0	0
Trehalose	0	+	0	0	+	0	0	+
Production of hemolysis of blood	0	0	+	0	0	0	0	0
Production of indol	0	0	0	0	+	0	0	+
Production of gas on carbohydrate	0	0	0	0	+	+	0	0
Reaction on litmus milk	0	Acid coagulation	0	0	Acid coagulation	Acid coagulation	0	0

* Strains 24 and 25 were isolated from patients who survived.
† A reaction of p_H 4.6 to 6 is indicated.
‡ Strain 11 fermented raffinase.
§ Strain 23 made all mediums alkaline except when fermentation occurred.

TABLE 3.—*Agglutination Tests*

	Titer of Agglutination with Given Antigen									
Serum	5	16	19	22	23	24	25	8	9	11
5	640	0	0	0	0	0	0	0	0	0
16	0	40	0	0	0	0	0	0	0	0
19	0	0	1280	0	0	0	0	0	0	0
22	0	0	0	640	0	0	0	0	0	0
23	0	0	0	0	640	0	0	0	0	0
24	0	0	0	0	0	320	0	0	0	0
25	0	0	0	0	0	0	80	0	0	0
8	0	0	0	0	0	0	0	320	80	40
9	0	0	0	0	0	0	0	80	160	40

the antigens. These results are in agreement with the biologic reactions in that strains 8, 9 and 11 belong to the same serologic group.

Pathogenicity for Animals.—Lesions were not produced in animals by subcutaneous, intramuscular, intraperitoneal or intravenous injections of pure cultures of the anaerobic streptococci.

Acute iridocyclitis could be produced in rabbits by injecting 0.1 cc. of the brain broth culture into the anterior chamber of the eye. In one day the cornea was bulging and the anterior chamber was cloudy. There was no change following the injection of a similar amount of sterile brain broth. Microscopic examination showed diffuse acute inflammation of the iris and ciliary body. By the end of seven days the eye became sterile and the inflammation began to resolve. Since even bacteria which commonly are accepted as saprophytes are capable of producing much more severe ophthalmia in rabbits than that produced by anaerobic streptococci, this experiment demonstrates the lack of virulence of the anaerobic streptococci more clearly than it does their ability to produce inflammatory lesions.

The injection of 1 cc. amounts of a broth culture of anaerobic streptococci into the testes of rabbits resulted in acute orchitis. From two to three days after the injection the testis was enlarged and congested. Microscopic examination revealed acute interstitial orchitis with beginning formation of an abscess.

Fatal acute cerebrospinal meningitis was produced in rabbits by injecting 1 cc. of a pure culture. Anaerobic streptococci were recovered from the meninges.

These results of the inoculation of anaerobic streptococci into animals emphasize the fact that such streptococci are practically nonvirulent for laboratory animals. There are several explanations for the almost universal failure to produce lesions in laboratory animals. In the first place, the virulence may have been lost in artificial culture. It was necessary to incubate all strains on laboratory mediums for some time in order to isolate anaerobic streptococci in pure culture or to identify them as anaerobic organisms. Marwedel and Wehrsig [20] produced gas gangrene in animals with a strain of anaerobic streptococci isolated from a war wound, but their strain rapidly lost virulence on artificial mediums. Secondly, lesions have been produced by others after they had injured the tissues of the animals with toxins (Weiss,[21] Prévot [14]). It is apparent from our studies that anaerobic streptococci are virulent when acting in symbiosis with other organisms, since they have been found so seldom in pure culture. Even in case 20 (ruptured duodenal ulcer) and in case 2 (pulmonary abscess) it was impossible to believe that anaerobic streptococci were solely responsible for the production of the lesions at their inception, although we failed after most careful efforts to demonstrate associated organisms at the time of death of the patients. The meninges, on the other hand, are rather vulnerable even to an

20. Marwedel and Wehrsig: München. med. Wchnschr. **62**:1023-1025, 1915.

21. Weiss, Charles: J. Immunol. **30**:394-396. 1936.

organism of low virulence. For that reason it seems probable that in cases 8, 9, 10 and 11 anaerobic streptococci alone were responsible for the lesions.

SUMMARY

The pathology and bacteriology of twenty-five cases in which anaerobic streptococci were isolated have been studied. The lesions associated with anaerobic streptococci in this series were divided into three groups: (1) ruptured intestine (chiefly gangrenous appendicitis), (2) chronic pulmonary infections and (3) acute cerebrospinal meningitis. In a biologic and serologic study of eleven strains, three were found to be related. All strains proved practically nonvirulent for laboratory animals.

General Review

THE EXPERIMENTAL NEPHROPATHIES

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(Concluded from page 121)

LESIONS PRODUCED BY RENAL DENERVATION

The relationship of the nervous system to the development of anatomic changes has been the subject of much study, particularly in recent years. The view has been promulgated that a nervous mechanism is in some way linked with the initiation and progress of tissue change. The precise nature of this association, however, is still widely debated.

Fröhlich was evidently the first to call attention to the association of a nervous mechanism with the process of inflammation. He observed the pathologic alterations in the mesentery of the sensitized frog following a shock dose of protein and described stasis of blood and plasma, dilatation of the blood vessels with edema of the surrounding tissues and migration of leukocytes. The changes were found in the presence of edema and swelling of the nerve elements, to which Fröhlich attributed the blame for the accompanying abnormalities. Besredka (1917) showed that an anesthetized animal sensitized to foreign protein did not manifest anaphylactic phenomena when subjected to a shocking dose.

Naegeli demonstrated the interesting fact that local cutaneous anesthesia obtained with 1 per cent procaine hydrochloride prevented the appearance of a positive Pirquet reaction in man—perhaps due to the obviation of nerve influence.

Klinge found evidence of hyperergic inflammation (increased plasma, stasis, hemorrhage, edema, hyaline swelling of connective tissue elements in the walls of blood vessels, necrosis, leukocytic infiltration) in the ears of previously sensitized rabbits, and with particular intensity in those in which the auricular nerves and cervical sympathetic nerve had been severed.

On the basis of their investigations of the effect of horse serum on the skeletal muscles of rabbits, Lasowsky and Kogan felt that damage

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of the nerves exerted an accelerating effect on the onset and course of hyperergic inflammation.

A study of the effect of renal denervation alone on the structure of the kidney was made by Seres, who reported parenchymatous degeneration.

Melzner denervated one kidney in each of 5 dogs and killed the animals from three to seventy days after operation. Two dogs killed early in the experimental period showed fatty changes and occasional necrotic zones in the tubular epithelium of both kidneys. Fatty changes, however, are normal. Each of the remaining animals showed a progressive interstitial lesion consisting of multiple foci of plasma cells, fibrosis and secondary atrophy of the glomeruli. In no instance did the author attribute the pathologic changes to denervation.

Pronounced degenerative changes in denervated kidneys with early widening of the vessels were reported by Nisio. In a subsequent study in which denervation was more completely executed, the kidneys showed early widening of vessels, which later disappeared.

A careful and well controlled study of this problem was made by de Gironcoli. One kidney in each of 5 dogs was totally denervated, and the animals were put to death from two to sixty days after operation. The changes included vascular dilatation, capsular thickening, interstitial edema and the presence of occasional erythrocytes in Henle's loops.

Sudden chilling and its vascular effects are obviously of great importance in this connection. That the renal vessels react to cold in the manner of those of the skin was demonstrated by Cohnheim and Roy, Wertheimer and Petersen and Mueller. The latter reported hematuria, cylindruria and albuminuria after sudden chilling. Mueller, Petersen and Rieder, and again Rieder, investigated the effect of renal denervation and concomitant bacteremia. Following intravenous injections of colon bacilli, they observed albumin, erythrocytes, casts and bacteria in the urine from intact kidneys. The urine from denervated organs, on the other hand, remained clear. On the basis of their findings these investigators "felt justified in implicating both the vasomotor status and the varying toxic agent in the production of the nephritic picture."

Roentgenologic examination by Milles, Mueller and Petersen (1931) of the kidneys of dogs after intra-arterial injection of bismuth oxychloride proved the denervated kidney to be generally larger and its vessels more dilated than its intact fellow.

Milles and his co-workers (1931) followed this with a report of the vascular changes induced in the intact and the denervated kidney of

the dog by rattlesnake venom. Roentgen studies following intra-arterial renal injections of bismuth oxychloride showed beading and obliteration of large portions of the vascular bed, particularly in the denervated kidney. Histologic preparations revealed congestion of glomerular tufts, dilatation of interstitial blood vessels with occasional rhexis and patchy degeneration of the tubular epithelium. Swelling of the vascular endothelium was seen in all kidneys but was more marked in the denervated organs. "It appeared that the nervous control of the renal vascular bed acts to protect that organ to some degree, at least, from certain noxious agents, and removal of this nervous control exposes the kidney to greater injury when toxic materials are injected and allows them to reach the kidney in marked concentration."

The same authors subsequently (1932) reported their observations on the response of the denervated kidney to epinephrine, snake venom and chilling. Denervation alone, as previously shown, produced vascular dilatation. Epinephrine exerted a vasoconstricting influence on the intact kidney and had no effect on the denervated organ. The toxic effect of snake venom was found more pronounced in the denervated kidney, and it was thought that possibly the absence of vasoconstriction permitted the poison to act on the tissue for a longer period of time. The intact and denervated kidneys presented similar changes, however—endothelial swelling, congestion and epithelial degeneration. In addition, the finer vascular bed of the denervated kidney was obliterated as a result of cortical edema. The same mechanism was believed to operate in animals dying of distemper. In these animals the denervated kidney revealed more pronounced abnormalities than the intact organ—"the normal kidney presumably protecting itself through vasoconstriction."

A short period of chilling produced vasoconstriction of vessels in the intact kidney. With longer periods of chilling, the results were inconclusive. Repeated daily chilling was claimed to produce obliteration of the finer vessels of the intact kidney, congestion, epithelial degeneration and endothelial swelling in the arteries and capillaries. Milles and his associates attributed the renal damage to a combination of autonomic dysfunction and the action of a toxic factor.

These studies were continued by Milles and Hardgrave, who repeatedly injected colon bacilli and pitressin (beta hypophamine) into dogs after these had been subjected to unilateral renal denervation. Three different groups of animals were studied. The first group (5 animals) received daily intravenous injections of colon bacilli. The results obtained were indefinite. Two dogs after a period of two months showed in the denervated kidneys distention of the glomeruli, tubular degenera-

tion and necrosis, a moderate degree of interstitial fibrosis and proliferations of the media and adventitia of small arterioles. One showed no changes in the denervated kidney. A kidney that had not been denervated showed changes similar to those described. Three of the animals showed the denervated kidney grossly diminished. One showed the denervated kidney enlarged. One showed no change in the size of the denervated kidney.

A second group (2 animals) received daily intravenous injections of pitressin, and a third group, daily injections of colon bacilli and pitressin. Both these groups revealed inconclusive anatomic pictures.

Kaiserling and Mathies reported the results of an extensive study of the relationship of nervous influence to the development of "hyperergic" inflammation. Renal denervation was performed in rabbits which had been previously sensitized to foreign protein (four injections of swine serum given at intervals of five days). Six hours after renal denervation, diuresis occurred with an absolute increase in sulfate, phosphate and urea. At this time, slight hyperemia of a few glomerular tufts was found. Twenty-four hours, and especially thirty-six hours, after denervation, the engorgement had spread to involve many glomeruli and the remainder of the vascular tree. A few erythrocytes and albuminous exudate within a few spaces of Bowman, hemoglobin and hyaline tubular casts were encountered. Tubular degeneration was slight, not progressive, and was found only twenty-four hours after nerve section.

The authors then injected swine serum into the denervated kidneys of the previously sensitized animals via the renal arteries. The intra-arterial dose never exceeded 2 cc. and was never repeated. Pronounced changes were reported as early as twenty-four hours after injection and included infarct-like foci, marked glomerular hyperemia, fibrinous thrombi in capillaries, albuminous masses within the capsular spaces and degenerative tubular changes. The small and medium-sized vessels later showed mural fibrinoid deposits with infiltration of erythrocytes and leukocytes. Kaiserling and Mathies remarked on the resemblance of their lesions to those of Masugi and Sato but emphasized the fact that the changes reported by Masugi were obtained only after repeated doses of larger quantities of serum and usually in combination with acacia or gelatin. Kaiserling believed, therefore, that renal denervation effected severe circulatory disturbances which conditioned the kidneys of the rabbits for the development of an antigen-antibody reaction. Injections of swine serum into the ear veins of rabbits after denervation resulted in changes similar to those described, while controls, in spite of repeated injections of protein, presented no histologic changes.

Comment.—Experimental evidence of the relationship of nerve influences to pathologic changes was discussed in the opening paragraphs of this section. The basic factors involved, especially in the kidney, may be interpreted as either vasoconstricting or vasodilating.

Renal denervation alone produces vasodilatation and perhaps slight degenerative changes of the tubular epithelium. The early response to a single exposure to chilling is constriction of the vessels of the intact kidney and subsequent vasodilatation; no effect is observed in the denervated organ. Repeated daily exposures to chilling induce obliteration of the finer vessels of the intact kidney, with epithelial degeneration and endothelial swelling in the blood vessels.

The intensification of lesions in denervated kidneys observed after the injection of snake venom, during distemper and after parenteral administration of protein suggests that the exaggerated pathologic response is to be attributed possibly to the more protracted period during which the noxa are permitted to remain in contact with the vascular endothelium. The results recorded after experimentally induced bacteremia, however, are at variance with such a conclusion and indicate, therefore, that the last word has not been said in this connection.

LESIONS PRODUCED BY PARENTERAL INJECTIONS OF PROTEINS AND NEPHROTOXINS

The study of the effect of proteins (apart from bacterial extracts) and cytotoxins (cytolysins) has also been pursued in an effort to ascertain what morphologic effects an altered tissue reactivity could induce. The results of such studies comprise the contents of this section. Since 1924 there have been relatively few investigations concerned with the determination of the effect of pure protein on the kidney. Studies of the cytotoxins have been, perhaps, more intensive.

Proteins.—Hepler and Simmonds sensitized rabbits by repeated intravenous injections of horse serum or 2 per cent solution of crystalline egg albumin. Five days after skin necrosis appeared, protein was introduced directly into the kidney. Injections were made either singly or as often as eight times at intervals of from five to six days, and hemorrhage and necrosis with marked inflammatory reaction were obtained in the kidneys of sensitized animals. The kidneys of control animals which received from one to eight intrarenal injections showed no changes other than those attributable to trauma, i. e., hemorrhage.

Cloudy swelling of tubular epithelium following the intraperitoneal injection of protein in rabbits was described by Pentimalli. In long-standing peptone intoxication the adventitial layer of the arteries at the junction of the cortex and medulla showed an increase of lymphocytes, monocytes and myelocytes.

Vaubel after repeated parenteral doses of horse serum reported what he considered an activation of the basic interstitial cellular elements in rabbits, i. e., nuclear swelling, cellular proliferation and the presence of giant cells and mononuclears (Hitchcock, Camero and Swift). In 25 per cent of his animals he found large interstitial foci of round cells and connective tissue elements with hyalinization of the neighboring glomerular tufts. In intravenously treated animals there were thrombi in the glomerular capillaries, occasional edema of Bowman's capsule and rare tubular casts. Contrary to Longcope's view (1913), Vaubel's opinion concerning these changes was that they were not in the nature of nephritis.

Letterer, on the other hand, was unable to obtain any positive results after the injection of protein (horse and rabbit serum) into the renal artery of the highly sensitized rabbit. He subsequently sensitized frogs by inoculation of the dorsal lymph sac with foreign protein and then studied the response of these animals to intravenous "shock" doses. He employed the method of a study of the living kidney originally devised by Richards. Capillariscopic examination showed transient stasis in the capillaries of the glomerular tufts, followed by ischemia. He believed that this finding supported Volhard's contention that the renal lesions of Bright's disease were based on a vasospasm of an allergic nature. Contrary to Volhard, Letterer thought that the vasospasm occurred not only in the vasa afferentia but also in the tuft capillaries and vasa efferentia.

Masugi and Sato reported the lesions produced in rabbits by means of repeated parenteral injections of protein (intravenous, subcutaneous or intraperitoneal routes). Three major groups of animals were studied. Rabbits which received repeated intraperitoneal injections of pulverized rat organs, blood serum or erythrocytes showed insignificant and scant histologic changes. In 23 per cent of the animals there was round cell infiltration of the perivascular connective tissue. In some of these, large basophilic cells with swollen nuclei and cytoplasm and occasional plasma cells were found in addition to small lymphocytes, which were considered allergic in nature.

In a second group to which were administered repeated intravenous or intraperitoneal and intravenous injections of protein, the kidneys similarly disclosed no noteworthy changes.

A third group (8 rabbits) was repeatedly given horse serum intravenously. In animals which had received large intravenous doses, the majority of the glomerular tufts were found altered. The changes consisted of enlargement and filling of the capillary loops with homogeneous "plasmatic" masses and ischemia. The walls of the loops were often delicate but occasionally edematous. The nuclei were increased

in number and size. Other changes were some swelling of the capsular epithelium, albuminous exudation and the presence of rare red cells within the capsular space. In some animals fibrin-like thrombi within capillaries and adhesions between the loops and capsule were described. The tubular epithelium showed degenerative changes, and the edematous interstitial tissue contained round cells similar to those already described. The intensity of the renal lesion was found to parallel roughly the height of the precipitin titer. The investigators, however, alluded to the possibility that the severity of the change in this group was associated with unilateral nephrectomy (performed for control study).

Masugi and Sato then studied the effects of the injection of protein directly into the renal artery of the previously sensitized animal. The procedure consisted of unilateral nephrectomy followed by the application of a clamp to the renal vessels of the remaining kidney and the injection of egg albumin. The protein was administered in combination with saline solution, gelatin or acacia in order to increase the viscosity of the serum. Controls showed albuminuria, hematuria, and cylindruria, and microscopically a few tufts presented a transient mechanical filling of the glomerular capillaries associated with slight regressive changes. Giant cells, cystic widening of the loops, masses of fibrin and occasional necrosis of loops were found but were not considered significant. In another group repeated injections were made into the renal artery several days after sensitization. Many tufts appeared altered. Thinning of the capillary wall followed by stasis was observed very early. Formation of fibrinous thrombi in the vascular units with ultimate endothelialization and recanalization, enlargement of endothelial cells, ischemia, thickening of the capillary walls and frequent infarction occurred later. The tubular epithelium showed hyaline droplet or vacuolar degeneration and here and there necrosis and some regeneration, while the lumens contained hyaline casts and erythrocytes. The alterations were most severe in the region of the infarcts but were present elsewhere also. In other organs, fibrinoid necrosis of the smaller arteries bearing a resemblance to periarteritis nodosa was described. The infarcts were attributed to severe stasis.

The authors felt that these changes were similar to those which resulted from the injection of nephrotoxin. Fahr (1936), however, contended that these changes were focal. The kidneys of sensitized animals were thought to differ both qualitatively and quantitatively from those of the nonsensitized and the intensity of change to vary directly with the sensitivity of the animal. On the basis of their findings the opinion was offered that nephritis is the result of a hyperergic response through direct action of noxa on vascular units. In a later paper Masugi summarized the histogenesis of "allergic" inflammation of the kidney. The

first response, it was believed, is attained through increased permeability of capillary walls and filling of the lumens with albuminous material and occasionally leukocytes. This is followed successively by endothelial proliferation, formation of fibrinous thrombi in the capillary lumens, swelling of arterioles, vacuolar or fibrinoid degeneration and finally vascular stasis which may ultimately lead to necrobiosis of the vessel wall and of the parenchyma.

The efforts of Staemmler to produce renal lesions with repeated injections of protein were entirely unsuccessful.

Cytotoxins.—Cytotoxins have been produced for almost every type of animal tissue. Surveys of the varieties and nature of these substances were made by H. Sachs, Pearce (1903), Sata and Friede.

Three different nephrotoxins have been described:

1. Autonephrotoxins—toxic substances which are believed to accumulate in the living animal following in situ unilateral ligation of either renal vessels or ureter or after renal trauma.
2. Isonephrotoxins—serums of animals treated as described under 1 when injected into a normal animal of the same species. Isonephrotoxins have also been claimed to result after the injection of renal material into another animal of the same species.
3. Heteronephrotoxins—serums produced by the introduction of renal substance into an animal of a heterologous species. The immune serum is then injected into an animal of the species from which the renal tissue was originally procured (e. g., rat kidney injected into a rabbit and the rabbit immune serum then injected into a rat).

It is beyond the scope of this paper to consider the possible relationship of the biologic investigation of the heterophil (Forssman) antigens and antibodies to the investigations on heteronephrotoxins. The reader is referred to the extensive reviews by Davidsohn and more recently by Buchbinder. The present survey is concerned only with the morphologic effects induced by the various immunologic substances. The presence of great quantities of the Forssman antigens in renal tissue is undoubtedly of basic significance in the development of renal lesions following the use of heteronephrotoxic serums. In all probability it is the high content of these antigens in renal parenchyma which accounts at least in part for the production of the rather constant and definite anatomic effects.

Lindemann was evidently the first to study the heteronephrotoxins. Guinea-pigs were given injections of macerated rabbit kidney. The immune serum produced in this fashion was found to be very toxic for rabbits; uremic manifestations, albuminuria and cylindruria were seen in the animals into which it was injected. Normal serum, on the other

hand, when injected into controls produced only transitory albuminuria. Histologically, after the injection of the immune serum there were severe tubular epithelial degeneration and occasional necrosis. The glomeruli showed no changes except a slight albuminous exudate within the subcapsular spaces.

The effect of isonephrotoxins was first investigated by Néfédieff. The serum of rabbits which had been subjected to unilateral ureteral ligation induced albuminuria and loss of weight when injected into animals of the same species. In 1 animal marked degenerative tubular changes were found.

Castaigne and Rathery, after unilateral ureteral ligation, reported that in the intact kidney there was partial to complete disappearance of the granules normally found in the cells of the convoluted tubules, while the nuclei of the cells remained normal or became pyknotic or fragmented. Similar abnormalities were produced by unilateral nephrectomy followed by intraperitoneal injection of renal substance.

Repeated subcutaneous injections of an emulsion of dog kidneys (from one to four injections at intervals of from four to eight days) were administered to rabbits by Ascoli and Figari. Another group of rabbits was subjected to unilateral ureteral ligation. The serum of the first group was injected subcutaneously into rabbits, dogs and occasionally guinea-pigs and rats. Following these injections, albumin, hematuria and casts appeared in the urine within a few days and usually persisted for weeks or months. Serum from the animals in which ureteral ligation was performed produced similar but less pronounced abnormalities. The animals given the serum of this group, however, were claimed to have hypertrophy of the left side of the heart. The authors believe that the harmful action which the autonephrotoxin produced by unilateral ligation exerted on the kidney on the intact side represented a mechanism similar to that which occurs in the human disease.

Employing a similar method, Albarran and Bernard found degenerative tubular changes in only 1 of 4 animals. In another series in which unilateral ureteral ligation had been done in rabbits and dogs, degenerative changes were never found in the kidney on the side not operated on.

A thorough investigation of this entire problem was undertaken by Pearce (1903). The use of autonephrotoxin and of isonephrotoxin yielded no remarkable effects. Pearce's first efforts to induce abnormalities with hemolytic heteronephrotoxic serums produced no changes except those attributable to hemolysis. On the basis of the evident disregard of hemolysins Pearce felt that the previously reported work was inconclusive.

An ingenious method was then devised in an attempt to obviate this hemolyzing factor. In order to rid them of their blood content, kidneys were perfused with saline solution before maceration for the purposes of injection. Rabbit serum prepared with unwashed dog kidneys when injected intravenously into dogs produced hemoglobinuria, cylindruria and albuminuria within twenty-four hours after injection, while casts and albumin persisted for several weeks. Microscopically, the outstanding changes were glomerular congestion and granular degeneration of the tubular epithelium with casts. On the other hand, rabbit serum prepared by means of washed kidney (perfused with saline solution) when injected into dogs never produced hemoglobinuria. Granular and fatty casts were frequent, however. Microscopically, fine hyaline thrombi in capillaries (possibly agglutinated red blood cells) were noted, associated with degeneration and fusion of endothelial cells and epithelial degeneration of tubules with fatty change of Henle's loops. The serum of dogs treated with heteronephrotoxic serum when injected into normal dogs induced cylindruria, albuminuria and fatty degeneration of the tubular epithelium. "Attempts to produce chronic lesions with heteronephrotoxic sera were unsuccessful."

In this connection another investigation is of interest. In 1904 Pearce produced periportal necrosis of the liver within twenty-four hours with serums prepared by injecting bile, blood or blood-containing organs into animals. Thrombi were observed within capillaries of the glomeruli, periphery of the lung and adrenal glands.

In the same year Pearce reported his studies on the specificity of cytotoxins. He showed that a serum prepared by repeated injections of red blood cells produced intense congestion of organs, cellular agglutination, hepatic necroses and fatty degeneration of renal tubules. In each case the changes were ascribed to the presence of hemo-agglutinin and hemolysins. Serums prepared by repeated injections of organs that had been perfused with saline solution were also strongly hemagglutinative and hemolytic in vitro but did not produce hemoglobinuria. Serums prepared by means of washed kidney, liver, pancreas and adrenal gland produced changes not only in the corresponding viscera but also in other organs. Nephrotoxin obtained by the use of washed kidneys produced albuminuria and cylindruria. Microscopically the cells of the convoluted tubules were swollen and granular, while the epithelium of the tubules in the medullary rays showed extensive fatty changes. Exudation into the glomerular space and hyaline thrombosis with degeneration of the glomerular tuft were also present. After the use of nephrotoxins the liver occasionally showed "extensive granular degeneration." The following conclusion was presented: "Aside from nephrotoxin, which has a distinct injurious action upon renal epithelium, the various cytotoxins

studied have no specific action in the morphologic sense. The action of the kidney itself cannot be said to be specific, for in addition to the nephrotoxic element another constituent (hemolysin) is present. The action of this serum might perhaps be described as special."

In a study of the action of nephrolysins on the kidneys of rabbits Le Play and Corpechat described enlarged kidneys, which microscopically showed interstitial connective tissue proliferation, dilatation of tubules, occasional formation of pseudocysts and scattered epithelial desquamation. Neither protocols nor photographs accompany the report. The descriptions correspond to spontaneous interstitial nephritis.

Woltmann investigated the comparative effects of a large group of cytotoxins. Sheep tissues were injected into geese for the production of an immune serum. With nephrotoxin he noted marked cloudy swelling and desquamation of the tubular epithelium. In spite of perfusion of the kidney preparatory to injection, the serum which he obtained was slightly hemolytic. He made the point that the changes after the administration of various cytolysins were not specific but varied only in degree. He felt, nevertheless, that nephrotoxin possibly produced the most constant changes. He concluded that "the histological changes which are observed in every case are secondary to the hemolytic factor present in all cytotoxic sera."

Marrassini claimed to have induced degenerative lesions in the tubular epithelium and in hepatic cells with serums prepared by injecting the nucleoproteid of rabbit liver into guinea-pigs. The changes produced with the guinea-pig immune serums were considered to be more marked than those obtained with the pure nucleoproteid mentioned.

Bierry and his co-workers investigated the effect of nucleoproteids of the kidney further. Bierry first prepared a serum by injecting the nucleoproteid of dog kidney into rabbits. The intraperitoneal injection of the rabbit serum into dogs induced albuminuria. Later Bierry and Pettit reported their studies of the serums produced with the nucleoproteids of the liver and kidney. They found albuminuria and cellular degeneration in the corresponding organs with each type of serum, although the serum obtained by repeated injection of the renal nucleoproteid proved more effective. Microscopic preparations revealed interstitial hemorrhage, glomerular congestion and granular degeneration of the tubular epithelium. The liver was the seat of congestion and of granular and vacuolar degeneration. Bierry and Pettit were of the opinion that the serum prepared with organ nucleoproteids affected other tissues in addition to that by means of which it was prepared. Bierry's later work with Mayer seemed to contradict this contention. They reported marked fatty vacuolation and granular degeneration in

the livers of dogs which had received a serum prepared from hepatic nucleoproteid, while the kidneys of these animals were reported to be intact.

A further study of the action of antinucleoproteid serums was reported by Beebe. Rabbits were repeatedly given gradually increasing doses of nucleoproteid prepared from dog kidney, liver and pancreas. Beebe felt on the basis of precipitin reactions that "definite antisera have been produced for the nucleoproteids of the liver, kidney and pancreas of the dog." He found that his serums were hemolytic and hemagglutinative in vitro in low dilutions, but in no instance did he observe hemoglobinuria. The serums were administered to small adult fox terriers either intraperitoneally repeatedly in small doses or intravenously in a single large dose. Nephrotoxin produced tubular casts with tubular dilatation and epithelial erosion. The glomeruli appeared normal. In the hepatic parenchyma it produced scattered vacuoles, while the pancreas, spleen and lymph nodes were usually normal. After the injection of hepatotoxin the only changes described were in the liver. The author concluded that "cytolytic sera having a high degree of specificity can be prepared from the nucleoproteids of tissues."

Pearce and Jackson repeated the work of Beebe, injecting into rabbits repeatedly serums prepared with the nucleoproteid and nucleic acid from renal and pancreatic tissues of dogs. The immune serums were then administered intravenously to dogs. Microscopically, there were swelling of the tubular epithelium, especially of the convoluted portions, slight fatty change and occasional epithelial desquamation. Glomerular changes were not observed. The liver showed granular and fatty degeneration. Serums prepared with nucleic acid (pancreas and kidney) produced less severe changes but a relatively greater degree of fatty change within the epithelium of Henle's loops. Pearce and Jackson ascribed these changes to the presence of hemolysin and hemagglutinin and, contrary to Beebe, were of the opinion that specific cytotoxic serums could not be produced by the injection of nucleoproteids, but that such serums induced a general toxic process which was especially active in excretory organs.

Sata presented the results of an extensive study of cytotoxins. Emulsions of guinea-pig kidneys were repeatedly injected into the peritoneal cavities of rabbits, and the immune serums were then administered intraperitoneally to guinea-pigs. In animals in which necropsies were made within five days after the injections—the acute stage—both the tubular epithelium and the hepatic cells presented hyperemia and hemorrhage, degeneration, necrosis and fatty change. In animals examined in the period from ten to twenty days after the injections there were cloudy swelling and fatty degeneration of the tubular epithelium and similar changes within the hepatic cells. In

animals put to death after several months there were renal contraction, cardiac hypertrophy and atrophy of the liver. Peculiarly, with hepatotoxin the only pronounced effect on the liver was seen in those animals put to death early and consisted of edema, severe fatty degeneration and cloudy swelling. In the animals killed at later periods the liver showed merely cloudy swelling, while the kidney showed hyaline degeneration, hemorrhage, atrophy, focal round cell infiltration and cyst formation (probably spontaneous interstitial nephritis). Hemolytic serums (prepared with erythrocytes) produced cloudy swelling and fatty degeneration of the tubular epithelium and focal liver necrosis. The animals all showed hemoglobinemia, hemoglobinuria, albuminuria, hyperemia and frequent visceral hemorrhages. Sata called attention to the hemolytic factor common to all toxins but believed that a relative specificity existed between each cytotoxin and the corresponding type of cell.

Pearce and Eisenbrey used a serum prepared by injecting into rabbits an emulsion of washed dog kidney cells. Microscopically, the kidneys of dogs given injections of this serum showed frequent casts, degenerative lesions of the tubular epithelium and sparse exudation of serum in the glomerular capsule. A hemolytic immune serum (prepared by injecting dog red blood cells into rabbits) produced dissolution of red blood cells, degeneration of tubular epithelium and serous exudate in the glomerular capsule.

Single injections of nephrolysin (obtained by repeated injections of an emulsion of unwashed dog kidney into rabbits) by Lüdke and Schüller produced albuminuria and cylindruria, loss of weight and weakness in dogs. Repeated injections induced an anaphylactic type of death. Normal rabbit serum produced only transitory albuminuria and no casts or epithelial cells in the urine. The animals treated with nephrotoxin also showed hemoglobinuria, leukopenia, blood in the stools and occasionally a picture of shock. Microscopically, there were (description limited to 3 animals) cloudy swelling and vacuolation of tubular epithelium and proliferation of the epithelium of Bowman's capsule. Fatty degeneration of the hepatic cells was present and was believed to be secondary to the kidney changes. "In all cases," the authors stated, "the sera had a hemolytic effect." Isonephrolysin produced anuria, albuminuria and cylindruria. In general, however, its effect was less marked than that of heteronephrotoxin. Autonephrolysin (prepared by ligating the ureter and artery of a rabbit and one to two weeks later injecting the serum of these into normal rabbits) induced changes similar but less marked than those caused by isotoxins and heterotoxins. Lüdke and Schüller denied the existence of an absolute specificity of nephrolysins.

Hyperemia of the glomerular tufts and the appearance of granular masses within the space of Bowman in rabbits which had received injections of isonephrotoxic serum were reported by Herzen. The tubular epithelium was stained unevenly, vacuolated, often desquamated, and the tubular lumens here and there contained hyaline or granular masses.

Wilson and Oliver repeated the work of Pearce and further determined the effect of serums which had been previously absorbed with erythrocytes. Dogs received single intravenous injections of anti-kidney serum obtained from rabbits. Lethargy, albuminuria, cylindruria and occasionally hematuria followed. The kidneys were usually enlarged, and the cortex was the seat of hemorrhagic foci. Histologic preparations showed focal necrosis of the glomeruli, fibrinous thrombi in the glomerular capillaries and often fibrin within the subcapsular spaces. Necrosis of the tufts was also present. Some showed a periglomerular exudate of round cells and polymorphonuclears. These changes were followed by hyaline transformation and obliteration. Although casts, hemorrhage into the tubular lumens, cloudy swelling and cellular desquamation were reported, the tubular changes were not considered striking.

Similar changes were obtained with absorbed serums except that the hemorrhagic phenomena were absent. A hemolytic and hemagglutinative serum (prepared by injecting a 20 per cent suspension of washed dog erythrocytes into rabbits) did not produce the aforementioned lesions. Further, the antikidney serum when absorbed with kidney suffered a distinct diminution of potency. Wilson and Oliver felt that the lesions were not the result of the hemolytic and hemagglutinative elements present in the serum and that nephrotoxic serum could be designated as specific.

The question of the specificity of the so-called nephrotoxic serums, however, has not been definitely settled. On the basis of a review of the literature and his own researches, Friede concluded that with the exception of hemolysin and spermatolysin the living organism does not produce a specific cytotoxin and that the injection of different types of serums might produce similar clinical and morphologic pictures.

The problem of producing renal lesions with nephrotoxin was not taken up again until 1929, when Masugi first reported his studies. Rat kidney and liver were used as antigen, and antiserums were obtained from rabbits which had received repeated intraperitoneal injections. The antiserums after inactivation by heat were injected intravenously into rats. Hydropic and hyaline droplet degeneration of the epithelium of the proximal convoluted tubules and absence of liver changes were reported after injection of the nephrotoxin. The hepatotoxin, on the

other hand, induced marked fatty degeneration of the hepatic cells and no renal abnormalities.

In 1934 Masugi reported the results of a more detailed study. The method of preparing serums was that of Pearce (described in an earlier paragraph). Rat organs were used as antigen, and rabbits were employed for the production of serums. Masugi described three degrees of change:

1. After four hours the glomeruli showed leukocytic accumulation, endothelial hypertrophy and hyperplasia and filling of the capillary lumens with homogeneous pale eosinophilic albuminous masses and fibrinous thrombi. Albuminous material was also observed in the capsular spaces and proximal convoluted tubules.
2. After twenty-four hours the endothelial hyperplasia became more significant. There were slight swelling of the tubular epithelium and some perivascular edema.
3. The most severe grade of injury, stasis, was thought to be due to vascular paralyses. Capillary engorgement, hemorrhage from the interlobular arteries, karyorrhexis and endothelial pyknosis were present. Necrosis and fatty degeneration of the vessel wall and cortical necrosis were also seen. The proximal portion of the vessel was dilated and showed fibrinoid degeneration and swelling. In the less severely affected kidneys only an occasional tuft showed changes. Specific hepatic lesions were described following the injection of hepatotoxin but will not be taken up here.

Since nephrotoxin also produced changes in the liver, Masugi felt that nephrotoxin possessed only a relative specificity and that the distribution of the lesion in the kidney was proportional to the dose of the toxin and its duration of action.

In a subsequent study in 1934 blood-free rabbit tissue was used for antigen in producing immune serums in ducks. Rabbits were then given from one to four intravenous injections, from 5 to 15 cc. of the serums prepared in ducks being given on each occasion. In 8 of 16 rabbits thus treated he observed clinical indications of kidney disease, which included albuminuria, hematuria, hyaline, waxy and granular casts, cellular elements and doubly refractile lipoids in the urine. Oliguria was found only occasionally. The blood pressure was usually elevated, while edema was not especially striking. The main renal lesions were claimed to be glomerular and diffuse. In the acute stage, Masugi described filling of the capillary loops with albuminous masses, endothelial proliferation, ischemia and a variable number of leukocytes. At a later stage, scattered areas of capsular epithelial proliferation with crescent formation were encountered. Proliferation and hyaline droplet forma-

tion of the tubular epithelium were found. Glomerular stasis was not observed in this group of animals. In the process of healing, the albuminous masses within the loops became granular and the capillary walls thinner and the circulation was restored. In the chronic stage, with hyalinization of the loops, regressive nuclear changes, proliferation of the capsular epithelium and crescent formation within the crescents, he frequently observed fibrinoid masses, hyaline droplets, erythrocytes, leukocytes and lymphocytes. Associated with glomerular abnormalities were tubular atrophy, interstitial fibrosis and infiltration with lymphocytes, plasma cells and leukocytes. The vessels at this stage became tortuous but were otherwise unchanged.

The remaining 8 animals of the group showed focal lesions, with evidence of healing in some. One had spontaneous interstitial "nephritis."

Hemprich also adopted the method of Pearce and of Masugi in his study of nephrotoxins. Rabbit organs were used as antigen and ducks as the intermediary serum donors. After intravenous administration of the serums to rabbits these early presented hyperemia of the glomerular and intertubular capillaries followed by swelling of the capillary endothelium. Enlargement of the tufts, appearance of plasmatic masses (serous exudate) within the capillary lumens, droplet degeneration of the tubular epithelium and hyaline casts within Henle's loops were seen in animals three days after the intravenous injections. Fibrinoid thrombi were observed in scattered capillary loops with a few giant cells and infrequently crescent formation and interstitial round cell accumulations. The author believed that the ischemia of the tuft was directly the result of a capillary cellular reaction rather than the effect of spasm of the vasa afferentia and that the earliest process was evidenced by hyperemia of the arterioles and glomerular capillaries. Fahr was in agreement with the contention of Hemprich that allergy was responsible for the development of glomerulonephritis, but he promulgated the view that two factors were concerned in its inception, namely, a favorable antigen plus a corresponding state of organ reactivity. (Compare this view with that of Klinge and Knepper and of Ahlström.)

Weiss investigated the heteronephrotoxins further, studying especially the early effects. Serums were prepared by repeated intraperitoneal injections of a mash of perfused rabbit kidneys into ducks.

Single or repeated injections of serum thus prepared were administered to rabbits. The kidneys were exposed by abdominal incision for the purposes of observation before biopsy or extirpation. In 2 of 5 instances cortical hemorrhages and color changes were noted soon after injection. Swelling and fusion of glomerular loops, occasionally serous

exudate between loops, cellular proliferation and infrequent tuft-capsular adhesions were seen in histologic preparations. Weiss felt that the primary capillary widening was due to paralysis of the wall with coincident static hyperemia. Later, owing to the inflammatory response, ischemia associated with narrowing of the capillary lumens supervened. The distribution of the change was reported to vary with the dose.

Contrary to the view held by Masugi, Weiss' opinion was that the renal lesion induced with heteronephrotoxin was not allergic since the lesions could be obtained with but a single injection.

Klinge and Knepper thought that nephrotoxin was not alone directly responsible for the development of hyperergic inflammation but considered "nephrotoxin" merely a significant factor concerned in conditioning the kidney of a sensitized animal for the initiation of such a process. The kidneys of rabbits that received multiple intraperitoneal or intravenous injections of emulsions of rabbit kidneys revealed no pathologic changes. On the other hand, animals given two or three repeated intravenous injections of swine serum in combination with intraperitoneal injections of rabbit kidney material presented stasis of glomerular loops, hyaline necrosis, occasional proliferation of capillary endothelium and crescent formation with degeneration of tubular epithelium. These lesions, however, were regarded to be of the Löhlein embolic type (Aschoff, Staemmler).

The so-called localization of allergic response was further claimed to have been observed by Knepper after the use of mechanical, thermal, pharmacologic and hormonal factors. The administration of posterior pituitary hormone in combination with horse serum in previously sensitized animals produced degeneration of the tubular epithelium with hyaline and fibrinous casts. The glomerular tufts were the seat of endothelial swelling, hyaline thrombi in capillaries and subcapsular exudate. The brain and liver also showed some vascular changes, so that a general vascular effect was probably primary.

It is of interest in this connection to recall the work of Marcus and Schmidt-Weyland, who claimed the production of gangrene in the ears of rabbits by injecting epinephrine and bacterial toxins. Shwartzman (1935) did not observe with epinephrine any augmentation of the primary thromboses and hemorrhage resulting from intravenous injections of the active principles concerned in the Shwartzman phenomenon.

Kashiwabara, using pure antikidney serum without hemolysins, obtained only tubular degeneration. With a hemolytic nephrotoxic serum renal lesions were produced which were similar to those described by Masugi and his associates. Kashiwabara, therefore, concluded that the lesions were produced by a polyvalent serum which contained both hemolytic and "allergic" factors.

Arnott, Kellar and Matthew perfused rabbit kidneys, emulsified them and injected the emulsion into ducks. The immune serums from the ducks were inactivated and repeatedly injected into rabbits. They produced cellular proliferation, swelling and ischemia of the glomerular tufts; later, fibrosis, hyalinization and crescent formation. Degenerative tubular changes and interstitial increase were also found. The lesions were of a focal type.

Comment.—Repeated parenteral injections of protein have been shown to produce marked glomerular and tubular changes (Longcope, 1913; Coulter and Pappenheimer; Hepler and Simmonds, 1928; Pentimalli; Letterer; Masugi and Sato) and occasionally to result in the reactivation of the mesenchymal elements of the interstitial tissue (Vaubel; Pentimalli; Masugi; Ahlström, and others). The results with isonephrotoxins and autonephrotoxins have been inconclusive. With heteronephrotoxins, on the other hand, marked abnormalities of the renal structural units have been obtained, their distribution and intensity varying with the dose of the injected material (Masugi; Weiss; Hemprich). The significance, however, of the hemolytic factor which seems to be common to all serums employed in these experiments has not been definitely shown. Neither has the precise relationship of the Forssman immune substances been determined in this connection. In spite of this, the pictures described indicate that the presence of a state of hypersensitivity is largely responsible for the appearance of the more widespread changes and seems to substantiate further the contention that tissue allergy is essential for the production of a diffuse nonsuppurative renal lesion.

RECAPITULATION

A perusal of the literature on experimentally induced lesions of the kidney reveals that diverse series of agents are capable of producing lesions. The lesions have been shown to be caused by chemical agents, bacteria or bacterial toxins, roentgen rays, renal denervation, excessive feeding of food and parenteral injections of proteins. The abnormalities reported following the use of chemical agents and irradiation have but a remote application to the human disease. Although glomerular changes have been produced by these methods, the value of such procedures resides more significantly in the pursuit of functional and toxicologic studies. The peculiar distribution of these abnormalities, moreover, does not permit a comparison with the human disease.

Reference has been made to the significance of the feeding experiments. Of much greater importance in regard to the etiology of Bright's disease is the rôle played by bacteria, bacterial toxins and proteins (including the so-called nephrotoxins). Intimately associated with this

question is that of the relationship of the nervous system with its vascular influences. Although diffuse glomerulonephritis satisfying all the criteria required for the diagnosis of the human disease has not yet been produced experimentally, evidence at hand points to the possibility that bacteria or the products of their growth and proteins may be responsible for the inception and development of glomerulonephritis. Whether such a process is a direct result or is accomplished through the production of an altered state of reactivity, i. e., allergy or hyperergy, has still not been finally ascertained.

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Laboratory Methods and Technical Notes

MODIFICATION OF ZIEHL-NEELSEN METHOD OF STAINING TUBERCLE BACILLI AND TISSUES IN PARAFFIN OR PYROXYLIN (CELLOIDIN) SECTIONS

Comparison of This Method with Cooper's Technic

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The staining of tubercle bacilli in tissues embedded in paraffin or in pyroxylin (celloidin) can be accomplished with the laboratory method generally used for the identification of the organisms in smears from sputum, but such stained preparations are quite unsatisfactory when it is desired to study the nature of the inflammatory process induced. A modification of the Ziehl-Neelsen staining method is presented here that makes possible the easy identification of the main cellular elements involved in tuberculous inflammation along with the acid-alcohol-fast bacilli.

PROCEDURES

Fixation of Tissues.—Different fixatives, such as solution of formaldehyde U. S. P. (1:10), Zenker's acetic acid solution,[1] Zenker's solution with solution of formaldehyde,[2] Held's fluid [3] and Bouin's solution [4] have given satisfactory results. A twenty-four hour period of fixation is sufficiently long, except for enucleated eye bulbs, with which it is necessary to prolong the period for complete penetration. Ordinary methods of embedding in paraffin and pyroxylin (celloidin) are used.

Staining of Paraffin Sections.—Slides with paraffin sections from 8 to 10 microns thick, mounted with Mayer's glycerin-albumin mixture (equal parts of egg white and glycerin), are placed on a Ranson warming plate at 40 C. for from

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1. The formula is as follows: potassium bichromate, 2.5 Gm.; bichloride of mercury, 5 Gm.; distilled water, 100 cc., and glacial acetic acid, 5 cc. (added just before use).

2. The formula is as follows: potassium bichromate, 2.5 Gm.; bichloride of mercury, 5 Gm.; distilled water, 100 cc., and neutral formaldehyde, concentrated 40 per cent solution, 10 cc. (added just before use).

3. The formula and method of use are:

Solution 1. Saturated aqueous solution of potassium bichromate, 40 cc.

Solution 2. Neutral formaldehyde, 40 cc., and glacial acetic acid, 10 cc.

Mix solutions 1 and 2 just before use and allow tissues to remain in fixative for from eighteen to twenty-four hours, then wash in running water for twenty-four hours before dehydration.

4. Bouin's fluid contains solution of formaldehyde U. S. P., distilled water, glacial acetic acid and trinitrophenol.

two to three hours or in an oven at from 37 to 40 C. for from twelve to twenty-four hours. They are then permitted to cool at room temperature, after which they are passed through xylene and alcohols of decreasing strength to water. The sections from tissues fixed with fluids containing Zenker's solution must be treated with iodine to remove the mercury bichloride precipitate, and the yellow sections stained with the trinitrophenol of Bouin's solution must be bleached by passing them through two or three changes of lukewarm tap water and then placed in distilled water. The sections are then ready for the staining procedures; if staining has to be postponed, they should be placed in 70 per cent alcohol, for if left in water for any extended period, as over night, they are in danger of becoming dislodged from the slide during the following treatment:

1. Cover the sections with Ziehl-Neelsen carbolfuchsin prepared as follows:

Basic fuchsin[5]	1 Gm.
Absolute alcohol	10 cc.
Phenol crystals	5 Gm.
Distilled water	100 cc.

Put these ingredients into solution with mortar and pestle. Filter. This stock solution will keep indefinitely.

It is best to place the slide on a staining rack with adjustable leveling screw controls in its base, permitting accessibility to the under surface of the slide for heating with an alcohol lamp. Heat is applied to the under surface of the slide until steam arises. *Do not boil.* The carbolfuchsin is allowed to cool and is then reheated in a similar manner and again allowed to cool.

2. Flood the slide and wash off the stain with distilled water.

3. Decolorize the sections in acid alcohol (3 per cent hydrochloric acid in 95 per cent alcohol) until they are white. It is best to use two Coplin jars with from 50 to 60 cc. of acid alcohol in each and remove the excess fuchsin stain in the first jar and complete the decolorization in the second jar.

4. Place in lukewarm tap water for from two to three minutes (to remove and neutralize the acidity of the decolorizing alcohol). Sections will assume a moderate pink color.

5. Rinse in distilled water—two changes.

6. Stain in Ehrlich's acid-hematoxylin staining solution for from five to six minutes. The formula for Ehrlich's acid-hematoxyl in stock solution follows:

Hematoxylin	2 Gm.
Glacial acetic acid	10 cc.
Glycerin	100 cc.
Absolute alcohol	100 cc.
Distilled water	100 cc.
Ammonium alum crystals in excess of saturation point	

This stock solution is allowed to ripen in the sunlight for two weeks or more. The container is left open with gauze tied over the opening to keep out dust.

The staining solution is made from the foregoing stock solution as follows:

Stock solution	1 part	8 cc.
Saturated aqueous solution of ammonium alum	2 parts	16 cc.
Distilled water	4 parts	32 cc.

5. A commercial dye marketed by Grübler under the name given.

Mix and filter:

7. Wash in lukewarm tap water for from two to three minutes.

8. Rinse in distilled water.

9. Counterstain with 1 per cent aqueous solution of methyl (not methylene) blue for fifteen seconds. As the stain ages the period of counterstaining must be prolonged. It is best to prepare 50 cc. of this staining solution and renew it as needed.

10. Rinse with distilled water.

11. Pass quickly through methyl alcohol—two changes.

12. Rinse in acetone.

13. Put in acetone—toluene, equal parts, for thirty seconds.

14. Put in toluene—two changes.

15. Mount in balsam, preferably neutral.

Staining of Pyroxylin (Celloidin) Sections.—The method can be applied with equal facility to the staining of pyroxylin (celloidin) sections. In 1933 Dr. Dewey Katz from the division of ophthalmology of Billings Hospital and the Max Epstein Dispensary of the University of Chicago Clinics presented a paper before the Concilium Ophthalmologicum at Madrid in which he described the application of this staining method in ocular conditions: Enucleated eye bulbs are fixed in Held's fluid. Pyroxylin (celloidin) sections of the eye, like those of other organs or tissues, are cut and placed in 80 per cent alcohol just before they are attached to the slides. A drop of Mayer's glycerin-albumin mixture is smeared on each slide. Sections are oriented properly on the slides thus smeared, covered with a lintless blotter and flattened by moderate pressure. Each of the pressed or flattened sections is covered with a film of oil of cloves which is allowed to remain until the pyroxylin (celloidin) becomes invisible. The oil of cloves is then poured off, and the slides are placed in 95 per cent alcohol. The slides are then transferred to clean 95 per cent alcohol and subsequently placed in a mixture of equal parts of absolute alcohol and ether, in which solution they are kept for several hours (over night if necessary). They are then carried through alcohols of decreasing strength to distilled water and are ready to be submitted to the first staining procedure, namely, that with carbolfuchsin. The staining method is applied as for paraffin sections.

Microscopic Examination of Stained Sections.—For microscopic examination of stained sections one should make use of some source of light other than the conventional microscope lamps with blue filters, as the excess of blue causes the acid-alcohol-fast bacilli to appear purple and not brilliant red. Excellent color contrasts can be secured by using a light filter made with equal parts of 1:100,000 aqueous erythrosin and 1:200,000 aqueous orange G in a flask used with the Leitz or Zeiss lamps. The tubercle bacilli appear stained a brillant red. The cell cytoplasm presents different tints of blue, while the nuclei are deep purple. The fibrous connective tissue also stains a distinct blue. Erythrocytes retain some of the red fuchsin stain.

ADVANTAGES OF THE STAINING METHOD

The advantages of this polychrome staining method for the identification of acid-alcohol-fast bacilli and related tissues lie in its rapidity and in the satisfactory differentiation of cells and tissue. As to its permanency, sections stained and mounted in neutral balsam six years ago and kept in covered slide boxes do not show any evidence of fading.

It is important, however, that if after mounting a drying procedure is used the slides should not be exposed to temperatures over from 40 to 45 C. for more than twenty-four hours. Prolonged exposure to higher temperatures causes decolorization of both bacilli and tissues.

DESCRIPTION AND COMPARISON OF COOPER'S METHOD

In 1926 Cooper [6] introduced a modification of the Ziehl-Neelsen staining method for the demonstration of tubercle bacilli in smears of sputum, and in 1932 Gay [7] reported most promising results from the application of this staining procedure to tissue sections. Knowing several investigators in the field of experimental tuberculosis who, like Gay, believe that the method discloses about 50 per cent more tubercle bacilli than other staining methods, I submit the details of the technic and an analysis of its coloring effects.

Staining Technic of Cooper's Method.—1. Sections brought to distilled water as in my method (page 265) are placed in a mixture of Ziehl-Neelsen's carbol-fuchsin, 48.5 cc., and 10 per cent aqueous sodium chloride, 1.5 cc., in a Coplin jar and allowed to stain for from two hours to all night in an incubator at 38 C.

2. Place the Coplin jar containing the sections in the icebox for thirty minutes to precipitate the stain.

3. Wash in tap water.

4. Stain with azure II-methylene blue solution [8] for from three to five minutes.

5. Wash in distilled water.

6. Differentiate in 95 per cent ethyl alcohol containing 1 drop of rosin (colophonium) in xylene for each 50 cc. of alcohol.

7. Dehydrate, clear and mount.

Gay [2] recommended fixation in Zenker's solution and embedding in paraffin. I find that the fixatives used in conjunction with my method of staining tubercle bacilli work equally well with Cooper's technic, and that material embedded either in paraffin or in pyroxylin (celloidin) may be used.

The color complexes rendered by this staining method are individual and combined red and blue. Basic fuchsin, methylene blue and azure II are chloride stain combinations. Azure II is a methylene-blue and methylene-azure complex. The carbol fuchsin stain contributes considerable red color to tissues, especially to fibrous connective tissue, and this color is not the most satisfactory background for distinction of tubercle bacilli stained a deeper red; the brilliant red of acid-alcohol-fast

6. Cooper, F. B.: Arch. Path. **2**:382-385, 1926.

7. Gay, D. M.: J. Lab. & Clin. Med. **17**:1131-1132, 1932.

8. The solution is prepared from the following:

(a) 1 per cent aqueous solution of azure II.

(b)

Methylene blue	1 Gm.
Borax	1 Gm.
Distilled water	100 cc.

Mix 3 cc. of a and 3 cc. of b and add 54 cc. of distilled water. Do not filter.

The diluted stain gives the best results when made up fresh and renewed for each day's staining. Stain in a Coplin jar.

bacilli is so sharp, however, that any one without a defect in color vision will have no trouble in identifying them where the tissue background is differentiated to a paler red color. One can reduce to a minimum the intensity of the fuchsin red color related to cells or tissues by counterstaining with the azure II-methylene blue solution for five minutes, and differentiating in the 95 per cent ethyl alcohol with rosin (colophonium) the shortest time possible. The maximum counterstaining time of ten minutes recommended by Gay has been found too long, as it gives a too opaque blue coloration. Well controlled counterstaining will allow brilliant red tubercle bacilli to be identified among cells and tissues presenting colors as follows: all nuclei different shades of blue; plasma cells, deep blue; smooth muscle cells, blue; Ehrlich's so-called mast cells, purple; macrophages, purple; giant cells, blue to light purple; epithelium, blue to purple, and fibrous connective tissue, reddish purple to pink. Not infrequently there is seen a large mononuclear cell with faint blue cytoplasm in which are several moderately large acid-fast granules stained deep red by the carbolfuchsin; these are not the so-called mast cells or eosinophils, and it is urgent to avoid confusing their red granules with tubercle bacilli in cross-section.

COMMENT

In animals in which tuberculosis has been experimentally induced, both staining methods display numerous tubercle bacilli. In a person with chronic tuberculosis, e. g., tuberculous salpingitis or appendicitis, it is often necessary to examine several sections stained by each method before a solitary tubercle bacillus can be found, and often neither method will demonstrate the organism. Since a careful comparison of the two methods as used on material with scanty tubercle bacilli slightly favors the Cooper technic, most sections to be studied are first stained by the quicker method which I have described, and if the results are negative, additional sections are subjected to the slower Cooper staining procedure for final study.

Case Reports

ACUTE ENDOCARDITIS PRODUCED BY BACILLUS PARATYPHOSUS B

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Paratyphoid infections have such a low mortality that but few reports of necropsies appear in the literature. In 1904 Wells and Scott [1] were able to collect but 5 fatal cases with postmortem examination recorded up to that time, and in 1915 Loele [2] found records of 56 cases. The greatest number of fatal cases occurred during the war, and Dawson and Whittington [3] reported in detail, with superb illustrations, the observations at necropsy in 15 cases of infection with B. paratyphosus B and 2 cases of infection with B. paratyphosus A in the British Army. In the First German Army, Kretschmer [4] reported necropsies in 1 case of infection with B. paratyphosus A, 10 cases of infection with B. paratyphosus B and 5 cases of probable infection with the latter organism. Herxheimer [5] reported necropsies in 20 cases of infection with B. paratyphosus B and in 8 cases of infection with B. paratyphosus A among German soldiers. Sternberg [6] observed an epidemic in the German Army with a mortality of 4.2 per cent in those infected with B. paratyphosus B and of 3.5 per cent in those infected with B. paratyphosus A; necropsies had been made in 75 of the former and 14 of the latter cases. There was no difference in the pathologic anatomy in the two groups. Suzuki [7] reported the observations at necropsy in 60 cases of paratyphoid observed in the Third German Army by Pick (54 of type B and 6 of type A). He mentioned 18 cases reported by Galambos. Vogelsang [8] reported 21 necropsies among civilians in Norway who died of infection with B. paratyphosus B between 1914 and 1932. D'Alesandro [9] reported the necropsies in 15 cases of proved or supposed paratyphoid in Italian civilians.

In but 1 of these 320 cases was endocarditis observed as a complication, nor was endocarditis observed in numerous other single necropsies and smaller series, indicating that, like typhoid, although a bacteremic disease, paratyphoid does not often lead to infection of the heart valves. Thus, Thayer [10] stated that in a series of 2,000 necropsies on typhoid

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1. Wells, H. G., and Scott, L. O.: J. Infect. Dis. **1**:72, 1904.
2. Loele: Ergebn. d. allg. Path. u. path. Anat. **18**:1, 1915.
3. Dawson, B., and Whittington, T. H.: Quart. J. Med. **98**:9, 1916.
4. Kretschmer, W.: Deutsches Arch. f. klin. Med. **178**:298, 1935.
5. Herxheimer, K.: Berl. klin. Wchnschr. **53**:648, 1916.
6. Sternberg, C.: Beitr. z. path. Anat. u. z. allg. Path. **64**:278, 1918.
7. Suzuki, S.: Virchows Arch. f. path. Anat. **250**:685, 1924.
8. Vogelsang, T. M.: J. Infect. Dis. **55**:276, 1934.
9. d'Alesandro, F.: Clin. med. ital. **58**:420, 1927.
10. Thayer, W. S.: Am. J. M. Sc. **127**:391, 1904.

in Munich, 11 (0.55 per cent) disclosed endocarditis, whereas in "nearly one hundred" necropsies on typhoid at the Johns Hopkins Hospital there were 3 cases of endocarditis. It seems probable that, corresponding with the apparently greater pyogenic tendency of paratyphoid infections, endocarditis may be somewhat more common in these infections than in typhoid, but there are too few reported necropsies to enable one to determine this conclusively, and but few cases of paratyphoid endocarditis have been reported in the literature.

Cumston,[11] discussing observations on paratyphoid made in the military hospitals of Lyon, Grenoble and Chambéry during the World War, said, "Twice lesions of acute endocarditis have been met with; one was a vegetating endocarditis of the aortic valves, the other a plastic endocarditis of the mitral and pulmonary orifices." Unfortunately no information is given as to how many necropsies yielded these 2 cases of endocarditis or as to the bacteriologic proof that the endocarditis was due to paratyphoid bacilli. Leschke,[12] in discussing rare causes of acute endocarditis, mentioned "paratyphosus Bacillen (Kaufmann: Tödlicher Verlauf unter hinzutretender pneumokokken Mischinfektion [fatal course with complicating pneumococcic mixed infection])." Kaufmann[13] included paratyphoid bacilli among rare causes of acute endocarditis without mention of specific cases.

Among Kretschmer's[4] 16 cases was 1 case of paratyphoid B infection of septicopyemic character without the usual intestinal lesions, in which verrucous vegetations were present on the aortic cusps, associated with miliary pyemic abscesses, chiefly in the liver and spleen. He called attention to the greater pyogenic tendency of paratyphoid bacilli as contrasted with typhoid, exhibited especially in this case and to a less extent in numerous other recorded cases, e. g., those of Dawson and Whittington.

The only other recorded case found was reported by Margreth,[14] in a patient whose blood gave positive agglutination with B. paratyphosus A at 1:200 and was negative to B. paratyphosus B. The necropsy disclosed verrucous vegetations on the mitral valves and acute meningitis. The only evidence of paratyphoid infection seems to have been the agglutination reaction, for cultures of both blood and spinal fluid during life were negative, and no mention is made of postmortem cultures of the valves or of anything else; therefore the identity of this as paratyphoid endocarditis cannot be considered as established.

In the report by Rathery and Ambard[15] on clinical observations on 1,088 cases of infection with B. parathyphosus B in which the condition was diagnosed clinically (in 314 by positive cultures; in 744 by agglutination only) no mention is made of any cases of endocarditis recognized either during life or after death (74 patients died, but it is not stated how many of these came to necropsy).

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11. Cumston, C. G.: New York M. J. **107**:603, 1918.

12. Leschke, in Kraus, F., and Brugsch, T.: Spezielle Pathologie und Therapie innere Krankheiten, Berlin, Urban & Schwarzenberg, 1923, vol. 4, p. 638.

13. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, ed. 9 and 10, Berlin, G. Reimer, 1931, vol. 1, p. 32.

14. Margreth, G.: Gazz. d. osp. **50**:1039, 1929.

15. Rathery, F., and Ambard, L.: Les fièvres paratyphoïdes B à l'hôpital mixte de Zuydcoote de décembre 1914 à février 1916, Paris, Félix Alcan, 1916.

In view of the evident infrequency of endocarditis from paratyphoid infection it seems desirable to record a case recently observed in the service of Dr. L. H. Sloan at the Illinois Central Hospital.

REPORT OF CASE

A car repairer aged 50 became ill Jan. 21, 1936. He went to a hospital in southern Illinois, where a diagnosis of pneumonia was made. As far as could be learned he had previously had no significant disease except possibly rheumatism. Later it was found that no other members of his family had been ill with a febrile condition at the same time as this patient. He had been accustomed to drink water from a brook running through the farm. There had not been any household epidemic of food poisoning. In the hospital he was delirious with a high fever for about a week, and his temperature failed to become normal after the more acute condition subsided. On March 12 thoracotomy was performed for suspected empyema, and pus was discharged for several days. From that time the patient had daily peaks of fever, with occasional chills and night sweats. He felt weak and had inspiratory pain in the left lower region of the chest together with an unproductive cough. There was considerable loss of weight but no jaundice.

He entered the hospital on April 10 because of continued fever, weakness and loss of weight and was seen by Dr. Wilber E. Post on April 20, who found petechiae of the skin and dulness at the base of the left lung. Dr. Post suggested the possibility of active bacterial endocarditis or of subphrenic abscess. Occasionally a systolic apical murmur was heard. The patient continued to have chills and fever with a daily rise of the temperature from 104 to 105 F. Laboratory study showed: leukocytes, 9,900; red blood corpuscles, 3,500,000; hemoglobin, 58 per cent; polymorphonuclears, 82 per cent; lymphocytes, 18 per cent. A blood culture made on April 11 was negative. The urine showed a trace of albumin and a few red and white cells. Examination of the sputum revealed nothing remarkable. An electrocardiogram made on April 20 revealed low voltage, axis deviation to the left, and a split QRS in lead III, interpreted as indicating marked myocardial damage. A blood culture was reported by the hospital laboratory as containing B. paratyphosus B. A Wassermann test was negative, but a Kahn test showed atypical precipitation. Blood sent to the Chicago Board of Health on April 20 showed positive agglutination for B. paratyphosus B from 1:40 to 1:500. Cultures of the urine showed neither typhoid nor paratyphoid bacilli. A Widal test made by the State Department of Public Health on April 17 was positive 1:80 for typhoid, negative for paratyphoid A and positive 1:320 for paratyphoid B. Tests for undulant fever and tularemia were negative. The Widal test was repeated on April 21 and was positive 1:160 for typhoid and 1:40 for paratyphoid B bacilli. A blood culture sent to the Chicago Board of Health on April 21 was reported as showing B. paratyphosus B; a stool culture made on April 23 was negative.

Death occurred on April 28, and necropsy was performed seven hours after death by Dr. C. E. Dunlap and me. The chief observations are indicated by the following anatomic diagnosis:

Paratyphoid B infection with acute vegetative mitral endocarditis; diffuse petechial hemorrhages of the skin, pericardium, epicardium, and bladder and intestinal mucosa; acute splenic swelling with large septic infarct of the spleen; gray pigmentation of Peyer's patches in the terminal ileum; slight acute embolic glomerulonephritis; focal acute myocarditis; infarct in the lower third of the right kidney; toxic swelling of the liver; early bronchopneumonia of the base

of the right lung; scar of old thoracotomy with a defect of the left tenth rib posteriorly; small encapsulated pneumothorax at the base of the left lung; atelectasis of the lower lobe of the left lung; chronic mesaortitis (syphilitic?); ossifying calcified nodule of the right epididymis (healed tuberculosis?); enlargement of the pancreas; calcified periappendical lymph node; atrophy and fibrosis of both testes; infarct of the skin of the left calf; desquamation of the soles of both feet.

The description of the heart was as follows: "The emptied heart weighs 510 Gm. and is correspondingly enlarged. On opening the mitral orifice a large shelflike vegetation is found attached to the middle of the auricular surface of the anterior cusp, extending out into the lumen. It measures about 2.5 cm. broad, 1.7 cm. wide and 1 cm. thick. On its under surface the vegetation is nodular and hemorrhagic, but on the upper surface, smooth and pale pink. On the posterior cusp is a superficial vegetative deposit over an area 1.5 cm. in diameter. There is slight thickening of the aortic cusps, but no evidences of old rheumatic disease. The remaining valves appear entirely normal. There is a little diffuse thickening at the base of the aorta and in the descending part of the arch, with a ring of wrinkled thickening at the level of the attachment of the aortic cusp. Microscopically the myocardium shows many small foci of acute inflammation with necrosis of muscle fibers and fragmentation of leukocytic nuclei. These often are located about fibrinous emboli, but no bacterial masses are found. There are a few small areas of replacement of myocardium by young fibrous tissue. There is marked increase in perivascular fibrous tissue, not incompatible with healed Aschoff bodies, but no definite rheumatic lesions are present. There are also much fatty infiltration and myocardial segmentation."

There were no ulcerations or other acute lesions in the bowel whether or not they had been present earlier in the course of the disease. The only change noted was that in the terminal ileum several of Peyer's patches showed slightly roughened surfaces with dirty gray pigmentation.

The most marked lesion outside the heart was in the spleen, which weighed 1,310 Gm. and was extremely friable; in the upper pole was a softened necrotic area of infarction 10 by 10 by 8 cm. This large softened area much resembled the condition pictured in one of the cases reported by Dawson and Whittington,[3] although there was no endocarditis present to explain it. There were also smaller suppurating infarcts in the kidney as well as slight acute glomerulonephritis; the latter condition has been observed in a few other cases of paratyphoid infection.

The postmortem bacteriology corroborated the evidence from the antemortem blood culture and agglutination tests in establishing this as a case of paratyphoid B endocarditis. Cultures from the heart valve, blood and spleen all contained the same organism, which was studied by Miss Helen Van Sant of the department of medicine of the University of Chicago and was found to agglutinate with paratyphoid B antiserum in a dilution of 1:2,560, with an antiserum for Salmonella aertrycke at 1:1,280, but not with paratyphoid A antiserum or an antiserum for Salmonella enteritidis even at 1:40. Fermentation tests made by Dr. Ben D. Chinn of the department of hygiene and bacteriology of the University of Chicago showed identical reactions in the antemortem blood cultures and the postmortem cultures from the vegetation on the heart valves, namely, fermentation of dextrose, sorbitol, rhamnose, maltose, xylose, mannite and dulcitol (after fourteen days) and no fermentation of lactose, saccharose, salicin, arabinose, trehalose and inosite. Tartrate medium was acidified; there was no production of indol or liquefaction of gelatin; the reaction on lead acetate medium was negative.

These observations indicate that the organism in this case belongs to the group of S. aertrycke, which formerly was included with Salmonella Schottmülleri as B. paratyphosus B (Jordan [16]). Since practically all the reported necropsies on paratyphoid infection antedate the more recent differentiation of S. aertrycke and S. Schottmülleri, it is impossible to know just what types of "paratyphoid B" organisms were present in earlier cases; hence this case will have to be included with those cases as "paratyphoid B" endocarditis although due to S. aertrycke. This is an exception to the fact that continued fevers resembling typhoid are usually due to Salmonella paratyphi ("type A paratyphoid"), S. Schottmülleri ("type B paratyphoid") and S. Hirschfeldii ("type C paratyphoid"), whereas S. aertrycke, S. enteritidis and more rarely Salmonella cholerae-suis are responsible for acute gastro-enteritis (Jordan).

16. Jordan, E. O.: A Textbook of General Bacteriology, ed. 11, Philadelphia, W. R. Saunders Company, 1935, p. 298.

News and Notes

University News, Promotions, Resignations, Appointments, Deaths, etc.—Simon Flexner, director emeritus of the Rockefeller Institute for Medical Research, has been appointed Eastman visiting professor at Oxford University for 1937-1938.

W. W. Brandes has been promoted to the position of associate professor of pathology in Baylor University, Dallas, Texas.

Amédée Borrel, bacteriologist at the Pasteur Institute in Paris and more recently professor of bacteriology and hygiene in the University of Strasbourg, has died at the age of 69 years. The genus of spirochetes known as Borrelia is named after him.

Stanley R. Benedict, professor of chemical pathology in Cornell University Medical College, died Dec. 21, 1936, aged 52 years. He originated the Benedict test for dextrose and other chemical tests of body fluids, and he edited the *Journal of Biological Chemistry* from 1920 until the time of his death.

Society News.—The annual meeting of the American Association of Pathologists and Bacteriologists will be held at Northwestern University Medical School, Chicago, Thursday and Friday, March 25 and 26, 1937. On the morning of March 26 there will be a symposium on the genesis of tumors. A joint session with the American Association of Immunologists will be held on the morning of March 25. The headquarters of the meeting will be at the Drake Hotel.

Mary Putnam Jacobi Fellowship.—This fellowship of $1,000 for one year is open to any woman graduate of an approved medical school. Applications for 1937-1938 should be sent to Dr. Annie S. Daniel, 321 East Fifteenth Street, New York City, before April 1, 1937. The recipient of the fellowship must give full time to investigation, preferably abroad.

Clinicopathologic Museum.—According to William Boyd (*J. Tech. Methods* **14**:10, 1935), the museum of the University of Manitoba Faculty of Medicine, Winnipeg, is in the process of transformation from a museum of morbid anatomy to what may be called a clinicopathologic museum. The most important single step in this departure is the introduction with the specimens of pictures of gross and microscopic sections, animal parasites, blood films, skin diseases, eruptive fevers, diseases of the throat and eye, temperature charts, roentgenograms, electrocardiographic tracings, a great variety of clinical states and the masters of medicine, both past and present.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Effects of Adrenalectomy and Hypophysectomy on Experimental Diabetes in the Cat. C. N. H. Long and F. D. W. Lukens, J. Exper. Med. **63**: 465, 1936.

Following hypophysectomy the pancreatectomized cat shows an alleviation of the diabetes comparable to that previously demonstrated by others in the dog. It is possible by various procedures to remove both adrenal glands and all the pancreas from a cat. Such animals have survived for as long as four weeks without the use of insulin, the average period of survival being eighteen days. Daily injections of cortical extract are necessary. By contrast, cats deprived of insulin by similar procedures but with the adrenal glands intact survive for a period of only four or five days. Adrenalectomized pancreatectomized cats show as striking an alleviation of the diabetes as do pancreatectomized cats which are hypophysectomized. This is expressed by the markedly decreased excretion of dextrose, nitrogen and acetone bodies compared with the excretion found in the pancreatectomized cat, as well as by the increased period of survival. Removal of epinephrine by denervation or demeduilation of the adrenal glands does not protect against diabetes in the pancreatectomized cat. The carbohydrate tolerance of pancreatectomized cats is not significantly increased by hypophysectomy or by adrenalectomy. The exceptions to this observation have all been in animals presenting varying degrees of spontaneous hypoglycemia. It is the opinion of the authors that the effects of hypophysectomy or of adrenalectomy on the diabetes due to pancreatectomy are due to diminution of the production of dextrose and acetone bodies rather than to resumption of the normal utilization of carbohydrate.

From the Authors' Summary.

Effect of Hysterectomy on the Ovary of Macacus Rhesus. T. H. Burford and A. W. Diddle, Surg., Gynec. & Obst. **62**:701, 1936.

The uterus was removed in five monkeys, including mature and immature animals. No impairment of ovarian activity could be detected. A postmortem survey of the ovarian follicles revealed no increase in follicular atresia in either group. One immature animal went on to definite sexual maturity 103 days after hysterectomy. It is concluded that hysterectomy in the type of monkey employed is without effect on the ovary except for purely traumatic sequelae. The monkey offers no evidence that the uterus contributes any endocrine influence on the ovary.

From the Authors' Summary. [Warren C. Hunter.]

Experimental Chronic Nicotine Poisoning. M. Staemmler, Virchows Arch. f. path. Anat. **295**:366, 1935.

The effects of chronic nicotine poisoning on the endocrine system, the gonads, the heart and the arteries were studied histologically. White rats were given daily subcutaneous injections of 1:1,000 and 1:10,000 dilutions of nicotine in doses of from 0.5 to 1 mg. The maximum period of the administration of nicotine was two years. Sixty-nine animals escaped intercurrent infections and other accidents; thirty-eight of these received nicotine for six months or longer. Hyperplasia of the medulla of the renal gland was observed in a considerable proportion of the animals. In twelve of thirty animals that had been subjected to nicotine for long

periods the process had led to the formation of adenoma of the medulla. No alterations were observed in the other endocrine glands. The testes of a few of the animals were atrophic; the author does not think the atrophy can be ascribed to the action of nicotine. The ovaries were not affected. No evidence of damage to the heart and blood vessels could be detected, and the other organs were normal. The young of nicotinized rats have appeared normal. O. T. SCHULTZ.

Pathologic Anatomy

SICKLE-CELL ANEMIA: A NEW CAUSE OF COR PULMONALE. W. M. YATER and G. H. HANSMANN, Am. J. M. Sc. **191**:474, 1936.

In two cases of sickle cell anemia there were hypertrophy and failure of the right side of the heart, due to numerous thrombotic occlusions of the medium-sized and small pulmonary arteries. One case showed pulmonary obliterating thromboangiitis, many of the vessels having patchy thrombosis with organization and recanalization or fresh thrombosis. In the second case were found medial hypertrophy and diminution in the diameter of the lumens of the arterioles of the lung; the medium-sized and small arteries had ancient changes, probably due to thrombosis and organization. Thrombotic phenomena were also observed in the spleen, the choroid plexus and the kidneys, and circulatory stasis was seen in the central nervous system and the extremities. In both cases the small arteries of practically all the organs had been subjected to prolonged severe strain, as evidenced by lengthening, tortuosity, hyalinization, hemorrhage into their walls and thrombosis. It seems probable that the distorted shape of the erythrocytes prevented their ready passage through the capillaries and thus led to thrombosis.

FREDERICK STENN.

CHANGES IN THE BONE MARROW IN AGRANULOCYTOSIS. R. C. DARLING, F. PARKER JR. and HENRY JACKSON JR., Am. J. Path. **12**:1, 1936.

The uniformity of the pathologic changes in the bone marrow suggests that agranulocytosis is probably a disease entity. Rapidly fatal cases show lack of maturation in the granular series and hyperplasia of the stem cells. Cases in which death took place after a longer period usually show hypoplasia of myeloid tissue with the coincident appearance of many plasma cells and lymphocytes. In no case were there obvious changes in the red cell series or any degenerative changes in the white cell series. Recovery is characterized by rapid development of the stem cells into myelocytes, metamyelocytes and polymorphonuclear neutrophils—a sequence of events which would appear to substantiate the contention of Fitz-Hugh and Krumbhaar and Custer that there has been an arrest of maturation. For this arrest of maturation the authors suggest the term "granulocytic anakmesis."

FROM THE AUTHORS' SUMMARY.

LESIONS IN THE AURICULOVENTRICULAR CONDUCTION SYSTEM IN RHEUMATIC FEVER. L. GROSS and B. M. FRIED, Am. J. Path. **12**:31, 1936.

Gross and Fried examined 110 human hearts in order to determine the nature and frequency of the lesions occurring in the node of Tawara and the bundle of His in rheumatic fever; 60 of these hearts represented active rheumatic fever; 25, inactive rheumatic fever, and 25, nonrheumatic conditions. It has been shown that in active rheumatic fever there occur various inflammatory and vascular phenomena within the horizontal conduction system as well as in the surrounding tissue. Even when studied in a few representative specimens from each bundle, the incidence of these lesions in the material showing an active process was approximately 66 per cent. It is probable that a study of more sections would have indicated a higher incidence. Very few of these lesions are specific or highly characteristic. The hearts in which the rheumatic process was inactive showed

few pathologic changes. This is in keeping with the functional differences observed as between these two groups. Attention has been called to the high incidence of inflammatory lesions in the collagenous extension of the septum fibrosum, and a discussion of the possible mechanisms concerned with the spread of the rheumatic infection to the bundle tissue is given. A description of the topographic relations of the horizontal conduction system in the human heart, together with the findings in 25 cases of nonrheumatic conditions, as controls, is also given.

FROM THE AUTHORS' SUMMARY.

TULAREMIA: REPORT OF THREE FATAL CASES WITH AUTOPSIES. ALAN BERNSTEIN, Arch. Int. Med. **56**:1117, 1935.

Three cases of tularemia are reported. The clinical course was characterized by sustained fever, tachycardia, dyspnea, prostration, delirium, signs of intoxication, extreme cyanosis, constipation, abdominal distention and evidence of pulmonary involvement. At autopsy in each case, caseous nodules, usually small but sometimes in coalescent groups forming larger necrotic areas, were found in lymph nodes, lungs, liver and spleen.

FROM THE AUTHOR'S SUMMARY.

SILVER CELLS (STEINER'S METHOD) IN MULTIPLE SCLEROSIS. N. KOPELOFF and N. BLACKMAN, Arch. Neurol. & Psychiat. **34**:1297, 1935.

The brains of eleven patients with multiple sclerosis, two patients with dementia paralytica and fifty-one patients with various morbid conditions have been examined for spirochetes and so-called silver cells. In ten of the brains from the eleven patients with multiple sclerosis silver cells were seen in the white substance bordering on the lateral and fourth ventricles, but no clearly defined spirochetes were found. Silver cells were especially numerous around the walls of the blood vessels. Both silver cells and spirochetes were found in the brains of the two patients with dementia paralytica. The controls comprised patients with a great variety of brain lesions, visceral disorders and systemic diseases. Only in one, a patient with congenital syphilis, were silver cells present together with numerous spirochetes. The authors thus confirm Steiner's contention that silver cells are to be found in spirochetal diseases, among which Steiner includes multiple sclerosis, but they "prefer to leave open the question of the incidence of spirochetes in cases of multiple sclerosis" until they have had an opportunity to examine fresher material.

G. B. HASSIN.

HISTOLOGICAL STUDIES OF THE ENDOMETRIUM DURING VARIOUS PHASES OF THE MENSTRUAL CYCLE. WALLACE E. HERRELL and ALBERT C. BRODERS, Surg., Gynec. & Obst. **61**:751, 1935.

From a study of the endometrium during various phases of the menstrual cycle certain new conceptions of the basic principles influencing regeneration of endometrium and a new classification of endometrial changes have evolved. Physiologic phases of the cycle are: an early proliferative stage lasting for from one to seven days after menstruation begins, a late proliferative period continuing through the fourteenth day, an early differentiative phase from the fifteenth through the twenty-first day and a late differentiative stage ending with the twenty-eighth day. Deficiency of humoral stimuli leads to pathologic persistence of the endometrium in any one of the normal cyclic phases, and if this fact is taken into account a diagnosis of pathologic hypertrophy or hyperplasia of the endometrium is not justified. Cystic endometrial glands are regarded as an evidence of physiologic senility.

WARREN C. HUNTER.

A Change in the Basophil Cells of the Pituitary Gland in the Syndrome Attributed to Basophil Adenoma. A. C. Crooke, J. Path. & Bact. **41**:339, 1935.

A hyaline change in the basophil cells of the pituitary gland is described which does not appear to be an expression of cell degeneration in the ordinary sense but is probably an expression of altered physiologic activity. It was found in slight amount in a few basophil cells of the anterior lobe in only 9 of about 350 pituitary glands from persons with various conditions in which the syndrome attributed to basophilic adenoma was present. Its presence in examples of the different conditions in which it was thus found was inconstant and exceptional. It was found in large amount in many basophil cells in the acini of the anterior lobe in all 12 available examples of the syndrome attributed to basophilic adenoma, a neoplasm of the thymus or a neoplasm or hyperplasia of the adrenal cortex. Analysis shows that this conspicuous amount of hyaline change was the only abnormality common to all 12 examples of the syndrome, and it is concluded that it is the abnormality of fundamental significance.

From the Author's Summary.

Siderosis in Cats. J. Taylor, D. Stiven and E. W. Reid, J. Path. & Bact. **41**:397, 1935.

The experimental effects on normal cats and the observations on what we have termed idiopathic siderosis in cats point to the likelihood of an intestinal lesion being intimately associated with the production of siderosis. If the entry of food iron exceeds the limits of assimilation and excretion, once deposition has started, the extent of accumulation of precipitated siderin is a matter of time and of the richness of the diet in iron. What cell conditions control the carrying of iron in some soluble form in contradistinction to its precipitation are unknown. Certainly we have found in the domesticated cat an animal which can suffer from siderosis as a pathologic condition, and if our idea is correct that malnutrition of the intestinal mucosa may lie at the back of a too easy entrance of food iron, the next step will be to determine the nature of the lesions in the idiopathic cases of siderosis. In these some irreparable nutritional disaster of unknown causation seems to have occurred. Whether the lesion is entirely or only partly in the intestinal mucosa and whether it is related to a foregoing period of hypovitaminosis, to an infection or to a combination of such conditions only future experiments can show.

From the Authors' Conclusions.

The Reticulo-Endothelium of the Spleen in Essential Thrombopenia. J. Wahlberg, Arb. a. d. path. Inst. d. Univ. Helsingfors **8**:206, 1935.

In three cases of essential thrombopenia the reticulo-endothelial cells of the spleen showed signs of increased activity, but whether this was primary or a reaction to the thrombopenia could not be determined. In the normal spleen the reticulo-endothelial cells may contain structures (chondriosomes, Golgi apparatus) which suggest internal secretion, and in thrombopenia these structures in the spleen present appearances that suggest hypersecretion.

Transformation of Nevus Cells into Sebaceous Gland Cells? G. Miescher, Arch. f. Dermat. u. Syph. **171**:119, 1935.

A smooth semiglobular slightly pigmented nevus was removed from the cheek of a 9 year old boy. In the basal epidermis were groups of nevus cells of the usual type. Immediately beneath this were small and large groups of cells, some of which were solid, but most of which showed the foam structure of sebaceous gland cells. Xanthoma is tentatively excluded, and the similarity of these cells to clear cells in unusual types of basal cell carcinoma is emphasized. The lesion supports the concept that basal cells possess possibilities of polyvalent differentiation and can form dendritic cells, sebaceous gland cells and neuroid cells. This is in contrast to Masson's latest theory, that nevus cells originate from the neural crest and wander to the epidermis rather than originate directly from epidermal cells.

S. W. Becker.

NEW FORMATION OF RETICULUM FIBERS. S. SÜMEGI, Frankfurt. Ztschr. f. Path. **47**:123, 1935.

In the liver of the rabbit with chronic chloroform poisoning destruction not only of liver cells is found, but also of reticulum fibers. Later, there is regeneration of liver cells and subsequently of reticulum fibers. These regenerated elements are, according to Sümegi, not the direct products of the cells but are formed "as the result of contact of two different colloid systems" (cell and matrix). The relative resistance of the regenerated liver cells to further chloroform poisoning is explained by a supposed production of antibodies. The conclusion is reached that reticulum fibers and connective tissue in general cannot be regarded simply as a supporting structure but should be considered as being endowed with vital activity.

OTTO SAPHIR.

IS ALL THE COLLOID FOUND IN THE MIDBRAIN OF HYPOPHYSEAL ORIGIN? E. SCHARRER, Frankfurt. Ztschr. f. Path. **47**:134, 1935.

Clinical and physiologic observations reveal that symptoms similar to those produced by extirpation or destruction of the hypophysis can also result from pathologic changes of the nervous substance of the tuber cinereum. This and other observations prove the presence in the midbrain of several of the hormones of the hypophysis. According to Colin, the hypophyseal colloid is transported to the midbrain along the nerve fibers and through the Virchow-Robin spaces and stored in the nucleus supra-opticus and nucleus paraventricularis. Scharrer, however, believes that not all of the colloid is transported into the midbrain, but that some of it is produced locally. He examined histologically a great number of brains of mammals, reptiles, amphibia and fish and found intracellular and extracellular colloid in the region of the nuclei mentioned. As the colloid was regularly seen in the midbrain in numerous animals of various species, it cannot be regarded as a product of degeneration or as an artefact.

OTTO SAPHIR.

GIANT CELLS IN CHRONIC MASTITIS. V. FABER and M. ROTTENSTEIN, Frankfurt. Ztschr. f. Path. **47**:173, 1935.

The authors believe that some of the giant cells frequently found associated with fat necrosis in chronic mastitis are true giant cells of endothelial origin but that others are derived from remnants of the epithelium of the destroyed glands. The hyaline globules that also are frequently seen in chronic mastitis are the products of degeneration and fusion of the cytoplasmic portions of plasma cells.

OTTO SAPHIR.

THE GENESIS OF MULTIPLE ENCEPHALOMALACIA AND OF ANGINA PECTORIS IN FOAM FORMATION. E. LÖFFLER, Frankfurt. Ztschr. f. Path. **47**:239, 1935.

Löffler investigated small but grossly recognizable foci of encephalomalacia located mainly in the basal ganglions and associated with severe sclerosis of the cerebral arteries. These arteries, in most of the cases, were markedly dilated rather than narrowed. He rejects spasm of the arteries as a cause of encephalomalacia without thrombosis because of the rigidity of the vessels. Changes in the blood pressure are, he thinks, not severe enough to cause encephalomalacia. The following explanation of encephalomalacia without demonstrable thrombi is offered: Because of the multiplicity and symmetry of the foci, it seems that embolic occlusions of the vessels must have occurred. The absence of proof of such occlusions is the fault of the methods of examination. For this reason an experiment was devised during which blood saturated with oxygen was subjected to sudden decrease of pressure. It was found that blood in a glass tube on sudden decrease of pressure becomes foamy. The foam possesses high viscosity and may act in the body like a solid thrombus, producing occlusion of the lumen, at least temporarily. Sudden variations of the pressure in the arteries of arteriosclerotic patients are frequent. The rigidly sclerotic arteries may be compared to the glass tube of the experiment. The most marked decrease of pressure occurs

in the small (end) arteries, the resulting foam thrombi may give rise to the small areas of encephalomalacia. The tortuosity and angular branching of the cerebral arteries likewise would be an important factor in the formation and slow resorption of foam thrombi. Conditions similar to those in the cerebral arteries are found in the coronary arteries in patients with angina pectoris. Löffler ventures the opinion that the cause of angina pectoris may also be attributed to foam formation.

OTTO SAPHIR.

ARCHITECTURE OF THE SMALL NODULE FORM OF CIRRHOSIS OF THE LIVER. R. RABL, Virchows Arch. f. path. Anat. **294**:605, 1935.

Of 300 livers with cirrhosis, material was selected from 80 for study of the lobular architecture means of serial sections and reconstruction. The parenchyma, the connective tissue framework and the vascular system were investigated separately and in relation to each other. The present discussion is limited to the small nodule, or hobnail, form of cirrhosis. The primary factor is held to be destruction or loss of tissue along the finer branches of the portal vein, the artery or the bile ducts. This leads to compensatory overgrowth of the parenchyma. The hyperplastic parenchymatous areas are subdivided by septums of supporting tissue. This process results in the formation of avascular adenomatous nodules. The succeeding fibrosis may be either intralobular or extralobular. The localization of this process does not seem to bear any definite relation to the cause of the cirrhosis, although in the majority of instances of cirrhosis ascribed to pathologic changes affecting the bile ducts the fibrosis was extralobular or perilobular. Subdivision of normal lobules by an ingrowth of connective tissue is a factor of minor importance in cirrhosis. The alterations in the circulatory bed brought about by the adenomatous hyperplasia lead to chronic edema and to rupture of the blood vessels into the lymphatics. New formation of vessels was observed only in the portal system; the central veins do not participate in this process.

O. T. SCHULTZ.

PNEUMONOCONIOSIS—IRON PIGMENT IN ANTHRACOSIS. I. M. PEYSAKHOWICH, and P. G. KOSTENKO, Virchows Arch. f. path. Anat. **294**:645, 1935.

DUST TRANSPORT AND LYMPH CIRCULATION IN THE LUNG. I. M. PEYSAKHOVICH and M. M. SCHEININ, ibid. **294**:648, 1935.

In the first of these two short papers the authors refer to the old view that anthracosis is due to a pigment of hematogenous origin and to the modern concept that it is due to inhaled coal dust. In 1928 Jousset returned to the older view. The present authors subjected dogs to an atmosphere laden with coal or silicate dust. The animals were killed and examined at various intervals. In both the experimental animals and the untreated controls the tracheal and bronchial lymph nodes were found to contain both coal pigment and iron-containing hematogenous pigment. The latter was not the product of any direct chemical or physical action of the inhaled dust but came from the erythrocytes broken down in the blood stasis caused by the fibrosis set up by the coal or other exogenous pigment. The exogenous and endogenous pigments bore a direct relation to each other in quantity. In the second piece of work the effect of impedence in the lymph circulation of the lung on the transport of inhaled dust was investigated. Unilateral pneumothorax was done in a series of dogs. Paresis of the thorax was produced in another series by alcoholization of the intercostal nerves of one side. The animals were placed in a dust-laden atmosphere for various intervals and examined. It was found that the inhaled dust was transported more freely to the hilus in the normally functioning lung than in the partly or completely compressed lung in which the lymph circulation was impeded. The fibrosis of spontaneous pneumonoconiosis interferes with the circulation of the lymph and thus decreases the rate of removal of the inhaled material from the lung.

O. T. SCHULTZ.

Microbiology and Parasitology

RESPIRATORY VERSUS GASTRO-INTESTINAL INFECTION IN POLIOMYELITIS. S. FLEXNER, J. Exper. Med. **63**:209, 1936.

The debated problem of gastro-intestinal versus respiratory infection in poliomyelitis has been restudied by several investigators with conflicting findings. Kling and Levaditi in Europe carried out experiments from 1929 to 1933 which led them to the conclusion that the digestive tract affords the virus a ready entrance into the body. They believe that the results of substituting Macacus cynomolgus for Macacus rhesus in tests support this point of view. Toomey in the United States has arrived at a similar conclusion, not by employing a particular species of monkey, but by using drastic measures of inoculation which insure that the virus makes contact with the unmyelinated nerve fibers embedded in the intestinal wall. Toomey's methods are so severe and artificial that they cannot be regarded as simulating a natural mode of infection. We have repeated the tests of Kling and Levaditi, but in a far more comprehensive manner, and, like Clark and his associates, who early repeated them, we have failed to confirm their findings. Indeed, we do not find M. cynomolgus and M. rhesus to differ in any essential way in their response to the presence of the virus of poliomyelitis in the body. M. cynomolgus does not respond to virus introduced into the stomach when contamination of the buccal and nasal cavities is avoided; it responds, as does M. rhesus, to virus injected directly into the intestine when the virus passes into the intestinal wall and makes the necessary contact with the nerve fibers. Monkeys of both species which have resisted infection after feedings of virus may be infected by nasal instillations of the same strains and in the same degree. From the experiments reported here we can reaffirm the conclusion previously arrived at by ourselves and confirmed independently by investigators in Europe and America, namely, that the only established portal of entry of the virus of poliomyelitis into the central nervous system of man is the nasal membrane, especially the olfactory nervous areas in this membrane.

FROM THE AUTHOR'S SUMMARY.

GENERALIZED VACCINIA IN THE CHICK EMBRYO. G. J. BUDDINGH, J. Exper. Med. **63**:227, 1936.

Chick embryos infected by the chorio-allantoic route with a bacteria-free strain of vaccinia virus show a general dissemination of the virus throughout the entire organism with the exception of the central nervous system. Quantitative estimation of the distribution of the virus in the various organs of the infected chick by cutaneous inoculation in rabbits' skin offers no evidence for a heightened affinity of the virus for special tissues. Histologic study of the lesions in the various organs demonstrates the focal character of the lesions, which apparently originate as perivascular infiltrations around the smaller blood vessels. No lesions could be demonstrated in the central nervous system proper. In the earlier stages of the disease Guarnieri bodies are clearly demonstrable in the cells of the epidermis and the squamous epithelium of the buccal mucosa. Inclusion bodies closely resembling Guarnieri bodies are demonstrated in all the lesions occurring in the various other organs. It was not possible to demonstrate conclusively the presence of Paschen bodies in the lesions of the internal organs by the Morosow method usually used for the demonstration of these bodies in the membranal lesion.

FROM THE AUTHOR'S SUMMARY.

ETIOLOGY OF RABBIT POX. L. PEARCE, P. D. ROSAHN and C. K. HU, J. Exper. Med. **63**:241, 1936.

A filtrable agent was isolated in seven cases of spontaneous rabbit pox by injecting a variety of tissues into the testicles of rabbits. The virus was transmitted for fifteen testicle to testicle passages in rabbits by means of Berkefeld V filtrates of emulsions of the testicular tissues. Unfiltered emulsions were more

potent than filtered. By icebox storage of the infected tissues the virulence was maintained for as long as 127 days. The pathogenic properties persisted under the conditions of animal passage for the nine months covered by these studies. The reaction produced in rabbits by the inoculation of the emulsions was of two principal types, the first of which has been described and analyzed in the present paper. From intratesticular injections, particularly those of testicular tissue, an acute fulminating and rapidly fatal condition regularly developed. The outstanding features were massive hemorrhagic orchitis with marked scrotal edema, fever and death within a week. The second type of reaction, which is taken up in the next paper of this series (*J. Exper. Med.* **63**:259, 1936), was observed under conditions in which the animal survived a week or longer. Occasional examples of this type occurred in rabbits of the so-called intratesticular series. The reaction was characterized by the development of a disease syndrome with a diversity of clinical manifestations which, it may be stated here for the sake of continuity, was indistinguishable from spontaneous rabbit pox.

FROM THE AUTHORS' SUMMARY.

SALIVARY GLAND DISEASE OF MICE. J. THOMPSON, J. Infect. Dis. **58**:59, 1936.

From experimental studies it is concluded that a focal mononuclear reaction together with inclusion-bearing cells in the salivary glands of apparently healthy mice indicates a virus disease. There is evidence to support this view: (*a*) Using the presence of an inflammatory reaction together with inclusions as an index, the condition was found to be transmissible to normal mice in series by the inoculation of bacteriologically sterile emulsions of affected glands. (*b*) Localization occurred only in the salivary glands following intraglandular, subcutaneous, intraperitoneal, intratesticular or intracerebral injection. (*c*) When the intracerebral route was used, localized encephalomeningitis developed in some of the mice. Histologically, the inclusion bodies in the cells of the salivary glands of mice resemble those of the guinea-pig, hamster, rat and mole. The salivary gland disease of mice after becoming established in a colony is transmitted for generations, as is the submaxillary gland disease of guinea-pigs. The condition has not been successfully transmitted to other common laboratory animals.

FROM THE AUTHOR'S CONCLUSIONS.

EFFECT OF DISSOCIATION OF STREPTOCOCCI ON THEIR FIBRINOLYTIC AND ANTICLOTTING ACTIVITY. R. TUNNICLIFF, J. Infect. Dis. **58**:92, 1936.

The fibrinolytic action of the hemolytic streptococcus appears to be associated with the virulent type forming smooth colonies. This power is lost as the colony becomes rough, and is regained as it reverts to the smooth form. Smooth and rough cultures of the viridans streptococcus and of the anhemolytic streptococcus do not show power to dissolve the normal fibrin clot, but smooth cultures completely prevent its forming. If the smooth form changes to the rough it loses its anticlotting action, and regains it if it reverts to the smooth form. The plasma clot of a patient recovering from a hemolytic streptococcus infection appears to resist the lytic action of the hemolytic streptococcus, and the plasma appears to resist the anticlotting property of both the greening and the anhemolytic streptococcus. The plasma of patients after an attack of measles or in a remission from the subacute endocarditis due to the greening streptococcus may become resistant to the anticlotting action of the greening and the anhemolytic streptococcus, but the clot does not resist the lytic action of the hemolytic streptococcus unless the patient is recovering from an infection with this organism.

FROM THE AUTHORS' SUMMARY.

FILARIASIS IN PUERTO RICO. F. W. O'CONNOR and C. R. HULSE, Puerto Rico J. Pub. Health & Trop. Med. **11**:167, 1935.

Filariasis thrives in regions marked by high temperature and high humidity and protected adequately from the wind. The plains and valleys of Puerto Rico

offer excellent ground for its spread. Of 2,098 persons examined, 503 showed the blood positive for microfilariae and 434 showed clinical symptoms of the disease. It is found in urban rather than rural communities and is most prevalent among the poor. The important vector is the mosquito Culex fatigans, which breeds in undrained pools, water barrels and refuse regions in numerous localities. The adult feeds between sundown and 11 p. m. Mosquitoes experimentally infected with Filaria Bancrofti do not show a heavy infection of the larval forms. The larval filariae in the mosquito host develop best in high humidity and at high temperature. Within twenty-four hours after the mosquito has imbibed blood the parasite may be found in the insect's thorax and about the seventh day is observed to curve on itself. When fully developed the larvae wander to the various organs and tissues of the mosquito. After penetrating the skin of man the parasites may develop locally or in the afferent lymphatics. Many of them reach the nearby lymph glands and are impeded in the afferent tributaries of the periglandular tissue or in the afferent branches of the nodes themselves. Some may develop within the glands. The parasites prefer lymphoid tissue; they may be found not unusually in the epididymis and spermatic cord. After growing to maturity the parasites of the two sexes meet in the afferent lymph vessels or in the sinuses of the glands.

Usually dilatation of a lymphatic occurs when the organisms lie within its lumen. The inflammatory change in the vicinity of living worms is not marked, and there is little eosinophilic and polymorphonuclear leukocytic response. The prominent cell is the small lymphocyte. Why the parasite dies within the lymphatic is not known, but soon after death it may become calcified; it may caseate and be absorbed by a giant cell, or infection may be associated. When calcification occurs small round cells predominate in the vicinity, and some giant cells are found near the worm; eosinophils are not present. When disintegration and absorption of the parasite have occurred considerable round cell and eosinophilic reaction occurs during the fibrosis, with the end-result of this degenerative change accounting for the complete obliteration of lymphatic vessels and disorganization of the lymphatic glands.

The blood of normal persons in endemic areas shows filariae. The microfilariae can traverse the wall of the lymphatic vessel and penetrate nearby blood vessels. Elephantiasis may or may not be associated with the inflammatory phenomena. With the beginning of the filarial attack there is a prompt polymorphonuclear leukocytic response with leukocytosis. The problem of filariasis in Puerto Rico appears to be difficult to solve.

FREDERICK STENN.

Immunology

TOXIC SUBSTANCES IN URINE AND SWEAT IN TYPHOID FEVER. N. STOLYHWO, J. Immunol. **30**:235, 1936.

Toxic substances capable of eliciting the Shwartzman phenomenon may be demonstrated in the urine and sweat of patients with typhoid fever. They are apparently excreted in amounts sufficient for elicitation of the phenomenon during the period of severe toxic manifestations. The facts seem to indicate that the phenomenon is elicited by the substances which are responsible for the severe intoxication accompanying typhoid fever. FROM THE AUTHOR'S SUMMARY.

THE EFFECT OF HETEROLOGOUS BACTERIAL PRODUCTS ON TUBERCULOUS ANIMALS. J. FREUND, J. Immunol. **30**:241, 1936.

The parenteral injection of products of colon or typhoid bacilli, like the injection of tuberculin, causes hemorrhages about tuberculous lesions and death in tuberculous guinea-pigs. The effect of products of typhoid bacilli is similar to that of products of meningococci in tuberculous rabbits. These bacterial products are injurious also to nontuberculous animals, causing intense congestion in some organs and, when employed in large amounts, death, but much less toxicity than in

tuberculous animals. The effect studied is probably due to the increased vulnerability of the blood vessels of the tissues containing tubercles. The observations do not cast doubt on the specificity of the tuberculin reaction.

FROM THE AUTHOR'S SUMMARY.

PRECIPITABLE SUBSTANCE FROM THE GENUS BRUCELLA. D. O. REITER, J. Infect. Dis. **58**:45, 1936.

Specific precipitable substances of polysaccharide nature were prepared from the three varieties of the genus Brucella by saline extraction, followed by precipitation with alcohol. These substances were feebly antigenic in rabbits but failed to give any of the usual reactions in chemical tests for proteins with the exception that under certain conditions the substance from strains A456 and S41-1 gave a doubtfully positive reaction in the biuret test. The specific substance from the abortus strain caused a typical anaphylactic reaction in a guinea-pig when 15 mg. was used as a sensitizing dose. The specific substances from the melitensis and the porcine strain failed to give a typical reaction. The preparations from the three varieties caused precipitation in antiserums for Brucella but not in antiserums for Salmonella. Antiserums for both the homologous and the heterologous varieties when absorbed with the precipitable substances from the three varieties showed a considerable reduction in agglutinin titers, indicating that the differences observed in the specific precipitable substances from the three strains may be quantitative rather than qualitative.

FROM THE AUTHOR'S SUMMARY.

BACTERIUM AERTRYCKE ENDOTOXIN. E. GRASSET, A. ZOUTENDYK and A. SCHAAFSMA, Brit. J. Exper. Path. **16**:454, 1935.

A recently isolated and highly virulent strain of Bacterium aertrycke which was responsible for an acute outbreak of food infection on a train has been the subject of study. A potent endotoxin was obtained by alternate freezing and thawing of concentrated killed emulsions. The toxic autolysates were rendered atoxic by the action of solution of formaldehyde (British Pharmacopeia) in 0.6 per cent dilution in the course of six weeks' incubation at 37 C. The antigenic properties of the detoxicated autolysates have been demonstrated by the immunization of mice, rats and horses. The hyperimmunization of horses gave serum possessing high specific agglutinating and flocculating properties, i. e., serum of considerable antibacterial and antitoxic value, as proved by tests in vivo and in vitro. The possibility of concentrating and refining the horse serum is now being investigated. The investigation tends to show that Bact. aertrycke antiserum may be utilized with satisfactory therapeutic results should the necessity arise.

FROM THE AUTHORS' SUMMARY.

THE HETEROPHILE HAPTEN AND THE SPECIFIC POLYSACCHARIDE HAPTEN OF THE SMOOTH FORM OF BACT. SHIGÆ. K. MEYER and W. T. J. MORGAN, Brit. J. Exper. Path. **16**:476, 1935.

The pure specific polysaccharide hapten of the smooth form of Bacterium Shigæ, which readily combines with the homologous antibacterial immune substance, is also able to neutralize specifically the hemolytic action of "Shiga" heterophilic antibody on the red cells of the sheep in the presence of complement. The relation between the power of this hapten to neutralize the heterophilic antibody and to give complement fixation with the homologous immune serum is the same for the crude and the purified preparations, and it remains unchanged during the destruction of these specific serologic properties by the action of acid, alkali or a species of Myxococcus. The constancy of this relation and the fact that treating the polysaccharide with antibacterial immune body greatly reduces its capacity to react with heterophilic antibody furnish strong evidence for believing that the heterophilic receptors and the specific bacterial receptors coexist in a single homogeneous polysaccharide molecule. It has not been possible by the action of

dilute acid or alkali to distinguish between the two serologically different types of receptors that are present in this hapten. Attempts to prepare a polysaccharide substance containing only heterophilic receptors by submitting the hapten to the enzymatic action of a species of Myxococcus were unsuccessful because of the inactivation of both types of receptors at the same rate. The fact that the chemically homogeneous specific polysaccharide hapten is able to form a specific combination with two entirely different types of antibody leads to the conjecture that a pure bacterial polysaccharide hapten may contain in each of its molecules two kinds of determinant receptors of diverse specificity.

FROM THE AUTHORS' SUMMARY.

STAPHYLOCOCCI FROM ANIMALS WITH PARTICULAR REFERENCE TO TOXIN PRODUCTION. F. C. MINETT, J. Path. & Bact. **42**:247, 1936.

The production of β toxin is a characteristic feature of hemolytic staphylococci from animals. Thus, of ninety-seven strains from various animal sources, mostly cow's milk, only five failed to form recognizable amounts of β toxin. Ten strains including eight from dogs, produced β toxin and only very small or undetectable amounts of α toxin. The staphylococci from dogs were also distinguished by their greater proteolytic power in culture. The α "unitage" of cow serum is generally below 7 and β "unitage" generally 0.45y or less.

FROM THE AUTHOR'S SUMMARY.

TETANUS TOXIN IN THE DIGESTIVE TRACT. R. REGAMEY, Ann. Inst. Pasteur **56**: 87, 1936.

Enormous doses of tetanus toxin given to guinea-pigs by mouth were tolerated without symptoms. The toxin disappeared from the stomach in forty-five minutes and from the intestines in an hour. It could not be recovered from the large intestines. The reduction in the toxin in the stomach was found to be due chiefly to adsorption. Experiments in vitro showed that bacteria, acidity and ferments had little or no effect. Noticeable attenuation of the toxin in the small intestines occurred in the absence of food and bacteria. Noticeable attenuation in the large intestines was attributed to adsorption and microbial flora. Bile was virtually without effect on the toxin.

FROM THE AUTHOR'S CONCLUSIONS.

IMMUNIZATION WITH MIXTURES OF DIPHTHERIA ANATOXIN (RAMON) WITH ALUMINUM HYDROXIDE. S. SCHMIDT, C. JENSEN and I. FJORD-NIELSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:276, 1935.

Rabbits were suecessfully immunized against the effect of diphtheria toxin by a single injection of a small amount of purified and concentrated toxoid to which aluminum hydroxide was added in the concentration of 10 per cent. Four weeks later the majority of the animals stood an intravenous injection of ten times the dose of diphtheria toxin that was fatal to untreated rabbits. The same procedure was used successfully for immunizing about 15,000 children in Denmark and Iceland, without any untoward complications. The technic of the preparation is described.

I. DAVIDSOHN.

INFLUENCE OF THE CONCENTRATION OF SERUM ON COMPLEMENT FIXATION AND PRECIPITATION. M. NIETHAMMER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:294, 1935.

Niethammer explains the well known occurrence in the same case of syphilis of a positive precipitation test and a negative complement fixation test by the inhibitory effect that the surplus of serum has on the complement fixation in the presence of small amounts of syphilitic reagins while under the same conditions the phenomenon of flocculation is not interfered with or is even enhanced. Diluting serum that gave a positive Wassermann reaction with serum that gave a

negative reaction served to illustrate the phenomenon. The inhibition of the complement fixation was less marked on diluting with serum that was heated to 65 C. It was demonstrated that the hemolytic properties of the serum were not a factor in the failure of the complement to become fixed. The complement fixation takes place with smaller amounts of serum than the precipitation.

I. DAVIDSOHN.

THE NORMAL DIPHTHERIA ANTITOXIN. S. ZURUKZOGLU and O. MÜNDEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:314, 1935.

Cultures were made from the noses and on occasion from the throats and tonsils of 100 horses of various ages. From one horse a typical diphtheria bacillus was isolated, and from another a bacillus that had the tinctorial and cultural properties of the diphtheria bacillus but was not virulent for the guinea-pig. While no definite conclusions are permissible, the results indicate that the possibility of a specific causation of the diphtheria antitoxin in horses cannot be denied.

I. DAVIDSOHN.

THE ANTIGENICITY OF CARBOHYDRATES. P. UHLENHUTH and S. REMY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:328, 1935.

Acacia has full antigenic properties, but after its purification by a procedure that removed a certain amount of the nitrogenous compounds its inoculation into rabbits was followed occasionally by development of small amounts of precipitin but of no other antibodies. The purified product was not anaphylactogenic in guinea-pigs. The experiments demonstrate the essential rôle of proteins in the antigenicity of acacia. Glycogen, even when mixed with hog serum, was not antigenic in rabbits.

I. DAVIDSOHN.

CHANGES OF THE HEMOLYTIC TITER AFTER REINJECTIONS OF ANTIGENS. P. GONZALEZ, M. ARMANGUÉ, S. ROMERO and R. SCHULZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:345, 1935.

Rabbits that had been given an injection of erythrocytes of the sheep or of an alcoholic extract of the kidney of the guinea-pig or of the horse with or without an inert adsorbent agent (for instance, kaolin) were then, after certain intervals, again treated with sheep erythrocytes or with alcoholic extract of sheep erythrocytes or of the kidney of the horse with or without kaolin. The results differed according to whether or not at the time of the reinjection the maximum hemolytic titer had been reached. A reinjection of sheep erythrocytes or of a mixture of the alcoholic extract with kaolin was usually followed by a preliminary drop and later by a rise of the hemolytic titer above the preinjection level. Following the reinjection of the alcoholic extract without the adsorber the hemolytic titer did not surpass the preinjection titer. The initial drop of the titer following the reinjection did not take place if the latter was administered while the hemolytic titer was on the ascent.

I. DAVIDSOHN.

Tumors

ISOLATION OF PURE STRAINS OF CELLS FROM HUMAN TUMORS. H. PINKUS, Am. J. Cancer **26**:521, 1936.

For experimental purposes it is desirable to have a pure strain of human tumor cells comparable to the pure strains of transplantable animal tumors. Of the material available, the gliomas were found to be particularly suitable for this purpose. Three pure strains of human malignant cells have been isolated and kept alive for from three to nine months.

FROM THE AUTHOR'S SUMMARY.

THE MACRONUCLEOLUS IN CANCER CELLS. W. C. MACCARTY, Am. J. Cancer **26**: 529, 1936.

In a malignant cell the nucleolus is larger in proportion to the size of the nucleus, the difference being great enough to permit differentiation from other cells in fresh tissues. The macronucleolus is "unquestionably diagnostic for malignancy, the only exception being neurocytes and ova, which are the only normal human cells containing large nucleoli."

SEASONAL INFLUENCE IN THE DEVELOPMENT OF TAR TUMORS IN MICE. L. KREYBERG and S. S. NIELSEN, Am. J. Cancer **26**:533, 1936.

In the strain of mice used in the experiments of the authors no seasonal influence was found to affect the development of benign or malignant tar tumors.

PRIMARY TUMORS OF THE PITUITARY GLANDS, OVARIES AND MAMMARY GLANDS IN A MOUSE. W. U. GARDNER, L. C. STRONG and G. M. SMITH, Am. J. Cancer **26**:541, 1936.

A pituitary adenoma, bilateral ovarian granulosa cell tumor and multiple mammary adenocarcinoma were observed in one mouse. No definite physiologic activity was observed that might be attributed to the pituitary adenoma. A uterine endometrial hyperplasia and a general cystic hyperplastic condition of the mammary glands were observed and ascribed to a hyperovarian state associated with the ovarian tumors. Though it is hazardous to state that mammary tumors are induced by a hyperovarian condition, the rôle of the ovarian hormone in the stimulation of the initial growth of the mammary glands must be a necessary factor.

FROM THE AUTHORS' SUMMARY.

THE RÔLE OF THE HYPOPHYSIS IN CANCER OF THE RAT. H. A. BALL and L. T. SAMUELS, Am. J. Cancer **26**:547, 1936.

Autogenous tumors produced by subcutaneous injections of 1, 2, 5, 6-di-benzanthracene in olive oil showed marked slowing of growth following removal of the pituitary gland as compared with tumors in control animals. The degree of retardation was approximately equivalent to that of the Walker mammary carcinoma 256 previously observed following removal of this gland.

FROM THE AUTHORS' SUMMARY.

OVARIAN TUMORS (GRANULOSA CELL CARCINOMA, ARRHENOBLASTOMA, DYSGERMINOMA, BRENNER TUMOR). E. NOVAK and L. A. GRAY, Am. J. Obst. & Gynec. **31**:213, 1936.

Granulosa cell carcinoma is relatively frequent and there should be a sharper lookout for it. While precocious puberty may be due to lesions of various other endocrine glands, the occurrence of this syndrome in association with an ovarian tumor should at once lead to the suspicion of granulosa cell carcinoma, and the suspicion will usually be proved correct. When curettage for uterine bleeding in a woman long after the menopause reveals typical endometrial hyperplasia, granulosa cell carcinoma should be suspected even if, as in very stout patients, it cannot be felt. If a tumor can be palpated, the suspicion becomes almost a certainty. During reproductive life the symptoms produced by granulosa cell carcinoma, aside from those of ovarian tumors in general, are like those characterizing the more common type of hyperestrinism. Menstruation is usually excessive, often irregular, sometimes normal, while long periods of amenorrhea are not uncommon. While in most cases the microscopic diagnosis of this carcinoma can be made from the morphology and the growth characteristics of the granulosa cells, many cases will be overlooked unless one is familiar with the many patterns produced by the connective tissue changes and by the apparent intermutability of the epithelial and connective tissue derivatives of the progranulosal ovarian mesenchyme.

Arrhenoblastoma should always be suspected if an ovarian tumor is found in a woman who, though previously of normal feminine type, exhibits symptoms of defeminization or actual masculinization.

Dysgerminoma, on the other hand, often occurs in sexually subnormal or pseudohermaphroditic patients, though the tumor has nothing to do with the production of such manifestations, which do not regress with the removal of the tumor, as they do with arrhenoblastoma.

The Brenner tumor of the pure or solid type is readily recognizable, but it must be remembered that the same histogenetic factor is concerned in the more and more frequently reported adenocystic fibroma as well as in at least a small proportion of serous and pseudomucinous cystadenomas.

The usual block or two made from the ovarian tumor for pathologic study is often not sufficient for a proper evaluation of its nature. Study of sections from many parts of the tumor is always desirable if a proper interpretation is to be made.

FROM THE AUTHORS' SUMMARY.

SUBCLINICAL ADENOMA OF THE PITUITARY GLAND. R. T. COSTELLO, Am. J. Path. **12**:205, 1936.

The small adenoma of the anterior lobe of the pituitary gland, instead of being a relatively rare pathologic curiosity, was shown to occur with considerable frequency—in 22.5 per cent of a series of 1,000 unselected cases, or in nearly 1 of every 4. Thus the anterior lobe is shown to act much like other glands of the endocrine system in its tendency toward adenomatous growth. It was shown further that, like adenomas in other organs of the body, adenoma of the anterior lobe occurs with increasing frequency in the higher age groups, and that it also apparently occurs in the same proportion of both sexes. It has also been shown that in the majority of cases adenoma of the anterior lobe, irrespective of type, is entirely benign and gives no recognizable clinical symptoms, and that it can be demonstrated only by rather detailed examination of the gland at autopsy. This would justify application of the term "subclinical adenoma" to the growth. It is entirely possible, however, that in some of the cases under the influence of an unknown stimulus adenoma may secrete a hormone or similar substance capable of producing clinical symptoms. The chromophobic adenoma has been seen to occur in about the same relative proportion of the series as do chromophobic cells in the average normal gland. However, the eosinophilic and the basophilic adenoma do not occur in the same relative proportions as their respective cells do in the average normal gland, and an adequate explanation for this has not been found.

FROM THE AUTHOR'S SUMMARY.

ADSORPTION AND ELUTION OF THE ROUS SARCOMA AGENT. E. M. FRAENKEL and C. A. MAWSON, Brit. J. Exper. Path. **16**:416, 1935.

An attempt has been made to purify the Rous sarcoma agent and particularly to separate the agent and its inhibitor by the selection of the most suitable form of alumina for adsorption and the use of buffers of varying p_H for adsorption and elution. The best adsorption materials were found to be the Wilstätter C and D aluminas, and the most potent eluate was one made from material adsorbed at p_H 6 and eluted at p_H 8.4. In general, the most potent eluates were those made at p_H 7.5 and 8.4. Since a considerable amount of the agent is left in the supernatant fluid during adsorption, and more is left in the residue during elution, no concentration of the agent was obtained by these means.

FROM THE AUTHORS' SUMMARY.

THE RELATION BETWEEN THE GROWTH RATE OF TAR WARTS IN MICE AND AUTOGRAFTS. J. C. MOTTRAM, J. Path. & Bact. **42**:79, 1936.

There was no close relation between the rate of growth of autografts and that of the warts from which they were derived. Different rates were sometimes observed when a number of autografts were made from a single wart. A careful

study suggests that such difference is not due to difference in extrinsic factors at the site of inoculation but to difference in the growth capacity of the cells inoculated. This with other evidence supports the view that the warts are often composed, not of like cells throughout, but of two or more groups of cells different in growth capacity as well as sometimes in histologic structure.

FROM THE AUTHOR'S SUMMARY.

THE BONE MARROW IN BROWN-PEARCE CARCINOMATOSIS OF THE RABBIT. J. W. ORR, J. Path. & Bact. **42**:105, 1936.

In twenty-nine rabbits with metastatic dissemination of the Brown-Pearce carcinoma hyperplasia of the bone morrow was found. The hyperplasia was predominantly leukopoietic, the erythropoietic marrow being increased in relatively few animals. The most marked grades of hyperplasia were associated with loss of erythropoietic tissue. The hyperplasia was frequently accompanied by anemia without qualitative alteration in the circulating erythrocytes. Various possible causal mechanisms have been considered, and it is suggested that the phenomenon may be due to continued absorption of the products of necrosis of tumor tissue.

FROM THE AUTHOR'S SUMMARY.

THE SKIN IN SARCOMATOSIS OF MICE. A. BESREDKA and L. GROSS, Ann. Inst. Pasteur **55**:402, 1935.

Inoculations of sarcoma under the skin give rise to tumors which evolve progressively to death, usually without metastases.

Intraperitoneal inoculations produce fatal peritonitis; at the level of the penetration of the needle into the peritoneum there is often a cutaneous tumor from which there may also be a similar tumor in the peritoneal wall. Fluid from the peritoneal cavity is very virulent by all routes of injection, especially the intraperitoneal route. In vitro, this fluid retains its virulence longer than an emulsion of the tumor.

Intracutaneous inoculations of sarcoma in massive doses give rise to tumors followed by rapidly fatal metastases in most of the animals tested; these intracutaneous tumors may disappear, while the metastases persist. Intracutaneous inoculations of small doses give rise to tumors which, after attaining the size of a coffee bean, are resorbed without death of the mice.

FROM THE AUTHORS' CONCLUSIONS.

MALIGNANT TUMORS FROM SOLAR IRRADIATION. A. H. ROFFO, Ztschr. f. Krebsforsch. **41**:448, 1935.

Roffo has succeeded in producing malignant tumors in white rats by prolonged exposure of the rats to sunlight. He finds that invisible portions of the spectrum with wavelengths of from 1,800 to 3,400 angstroms, are responsible and that it is their intensity rather than that of visible light which is the determining factor. From seven to nine months was required, with development of the tumors on naturally hairless or artificially depilated areas of the body. The irradiation was accompanied by a progressive increase in local cholesterol—a change similar to that noted by him on unclad surfaces of the human body, and he believes that this is important in cancerigenesis, through photodynamic effects of the cholesterol.

H. E. EGGERS.

THE CONNECTION BETWEEN ERYTHROBLASTOSIS (HEMOCYTOBLASTOSIS), MYELOSIS AND SARCOMA IN CHICKENS. J. ENGELBRETH-HOLM and A. R. MEYER, Acta path. et microbiol. Scandinav. **12**:352, 1935.

The occurrence of different types of disease in chickens carrying one strain of leukosis (e. g., myelosis and erythroblastosis, erythroblastosis and sarcoma, etc.)

and the distribution of the different types in the passages of one strain make it necessary to assume that all types of disease in one strain are produced by the same agent. For explanation of the change of type in a strain of leukosis we may imagine that the agent is modified by passage through an organism which is particularly disposed to development of a definite form of leukosis. If this hypothesis is correct, our experiences suggest that a great majority of chickens are disposed to have erythroblastosis and relatively few to have myelosis. As to the change of type between leukosis and sarcoma, the method of inoculation is an important factor, as intravenous inoculation most often results in leukosis while intramuscular or subcutaneous inoculation more frequently gives sarcoma.

Consideration of the relationship of the cells attacked in leukosis and sarcoma appears to offer a possible explanation of these conditions. An agent aiming at an immature cell—e. g., the hemohistioblast—is able to produce hemocytoblastosis as well as endotheliosarcoma and fibrosarcoma, merely through the hemohistioblasts' own developmental possibilities. If, on the other hand, the agent is aimed at the hemocytoblast, the result can be only leukosis (of one type or another) and not sarcoma. Accordingly, chicken leukosis, endotheliosarcoma and fibrosarcoma are three coordinate morbid processes.

We consider the agent itself as a product of the attacked cells, a product that can be reproduced only by the living cell. When introduced into a normal organism, the agent can induce tumor properties in the same kind of cells as the one from which it originated and make these cells reproduce the agent.

The agent appears to occupy an exceptional position in the great group of ultravisible viruses and is possibly to be regarded as a representative of a rather independent group of producers of disease—a group that has not received much attention hitherto—which might be called "endogenous agents."

FROM THE AUTHORS' SUMMARY.

Medicolegal Pathology

THE CENTRAL NERVOUS SYSTEM IN ACUTE METALLIC POISONING. A. ESSER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **25**:1, 1936.

A tremendous amount of information is packed into tables in this article, which deals with metallic poisoning in the spinal cord, medulla, brain stem and cerebrum. The effects of strontium, barium, magnesium, aluminum, thorium, thallium, zinc, cadmium and mercury were studied. Dosages are recorded for instances of ingestion of these substances by accident or with suicidal intent. In the spectrographic investigations Esser employed condenser currents, the electric spark and the high frequency methods.

G. J. RUKSTINAT.

A HEREDITARY DEFECT OF THE N AGGLUTINOGEN. V. FRIEDENREICH, Deutsche Ztschr. f. d. ges. gerichtl. Med. **25**: 358, 1936.

In a proceeding involving a question of paternity Friedenreich encountered an apparent exception to the laws of heredity of the agglutinogens M and N of Landsteiner and Levine. The mother's blood cells seemed to belong to type M and the child's to type N. Reexamination revealed that the mother had a very weak N agglutinogen in her blood cells. Forty-two other persons belonging to the family of this mother were tested, and thirteen were found to possess the weak N receptor. Friedenreich concludes that there are two sorts of N receptors, N_1 and N_2, of which N_1 is the more common and stronger agglutinogen, and dominant in its inheritance over N_2. Except for the exceeding rarity of occurrence of the agglutinogen N_2, this resembles in many respects the existence of two sorts of A receptors, A_1 and A_2, in human red blood cells.

A. S. WIENER.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

May 14, 1936

ESMOND R. LONG, *President*

HERBERT R. RATCLIFFE, *Secretary*

TUMOR IN A TADPOLE. BALDUIN LUCKÉ.

The tumor occurred in a tadpole of the green frog (Rana clamitans). It was located on the dorsal fin, parts of which were replaced by the growth, and was approximately as large as the head of the tadpole. Histologically the tumor had the structure of myxosarcoma; in several areas it had invaded and destroyed the upper muscle of the tail. No inflammatory reaction was present. The growth corresponded in every way to a true neoplasm.

Tumor in a larval amphibian has hitherto not been recorded. Such tumors would furnish exceptionally suitable material for certain experimental studies.

BONE MARROW IN THE ANEMIAS AS STUDIED BY STERNAL BIOPSY. R. P. CUSTER.

Biopsy of the sternal bone marrow has been practiced with increasing frequency at the Philadelphia General Hospital during the past four years and has served (1) to give the patient the benefit of more accurate diagnosis and prognosis, (2) to afford the attending clinician firmer ground on which to base therapeutic measures and (3) to make possible a more adequate catalog of the various disorders of the blood-forming organs and an accumulation of further data for the study of these disturbances.

A button of bone 1 cm. in diameter is removed from the ventral table of the sternum by trephine under local anesthesia; imprints and streaks are stained by Pappenheim's May-Grünwald-Giemsa method. The button is fixed in Helly's fluid, decalcified in formic acid-sodium citrate solution, sectioned in paraffin at 4 microns and stained by a modification of Maximow's azure II-eosin method. A differential count of 500 cells is made by a method previously described (Krumbhaar, E. B., and Custer, R. P.: *Am. J. M. Sc.* **189**:630, 1935).

Biopsy should be performed whenever possible before treatment of any kind has been instituted, especially the administration of liver. This is well shown by the example of early remission in pernicious anemia in which the patient was given a single dose of liver extract parenterally two days before biopsy; striking changes in the marrow precede changes in the peripheral blood, a reversion from massive megaloblastosis with imperfect erythropoiesis to relatively normal maturation of the erythron occurring.

While a scattering of promegaloblasts appears in the marrow in practically any type of severe anemia, erythropoiesis is essentially of the so-called normoblastic type in all varieties considered except pernicious anemia in relapse; in aplastic anemia a great gap lies between stem cells and late nucleated red cells, the few megaloblasts being confined to tiny foci of attempted regeneration. In sickle cell anemia erythropoiesis is normal save for a very occasional sickled normoblast.

Even though the results of studies of the marrow may occasionally be inconclusive, they are much less often so than are those of studies on the peripheral blood alone, and the practice of sternal biopsy deserves wider use than it is now accorded. Emphasis is again laid on the value of carefully prepared sections in conjunction with smears, as opposed to smears obtained by simple sternal puncture.

INFLUENCE OF THYMUS EXTRACT ON CERTAIN ENDOCRINE ORGANS OF THE RAT. J. H. CLARK.

By means of "three dimension" graphs illustrating age-weight-gland relationships it can be shown that thymus-treated rats differ quite uniformly from control rats in body size with respect to age. Each of the endocrine glands studied in the treated rat also differs in size from that of the control. This increase in the size of the gland is not proportional to the increase in body weight, some glands being greatly in excess of the estimated sizes, and others, much less. The thymus is consistently larger for the age-weight controls, and this persists throughout life. The adrenal gland is slightly larger for age controls but not appreciably for weight controls. No histologic differences were observed in the adrenal or in the thyroid gland. The thymus, however, appeared histologically to be overpopulated with lymphocytes, which tended to obscure the junction of the medulla and cortex. Hassall's corpuscles were less easily distinguished, seemed fewer and were composed of smaller numbers of reticular cells than normal.

In addition to greater rapidity of growth there was also increased sexual precocity in thymus-treated rats. The most marked and significant changes occurred in the testes and ovaries. The testes descended into the scrotum in thymus-treated rats within from two to three days instead of the normal thirty-one to forty days. They were larger and differentiated into the mature type, with lumens in the tubules about the tenth day (normal, within from thirty-one to forty days), and spermatids appeared about the twenty-fifth day (normal, fortieth day). Even greater precocity in sexual development and differentiation was noted in female rats. The vagina opened as early as the sixth day (normal, from the fifty-fifth to the sixty-second day), and corpora lutea were observed in sections of the ovaries as early as the ninth day (normal, from the sixty-second to the sixty-fourth day).

The pituitary gland in the thymus-treated rat seemed heavier than that in the age-weight control, more so in the younger than in the more mature animal. Pituitary glands from treated and control rats showed differences in the number and type of cells present, viz., acidophil, basophil and chromophobe. Cell counts made on the pituitary glands in each group, both male and female, at five day intervals from birth to 45 days of age confirmed this impression.

Cell counts were made on about seventy pituitary glands, equally divided between treated and control, male and female animals. The same procedure (total number of cells counted in different sections of the same gland, etc.) was used with each rat in each group. The data collected suggest that an increase in the acidophil cell content of the pituitary gland occurred in the thymus-treated rat exhibiting marked precocity in growth, i. e., body weight out of proportion to age. The same suggestive relationship (acidophil count of pituitary gland and body weight, regardless of age), however, seemed to exist in the control rats, as shown by comparative protocols of animals of the same age but different weight. From birth to about 15 days of age the test strain showed a 60 per cent increase (19 as compared with 11.9 per cent) in acidophils over the controls. The percentages of acidophils in both strains approached each other after this period. The difference in the percentages of basophils of the two groups while small (3 per cent as compared with 1.6 per cent) suggested that the sexual precocity in thymus-treated rats may in some way be concerned with this.

Further corroboration of this supposition is suggested by correlation with the histologic evidences of sexual precocity observed in microscopic sections of testes and ovaries or in vaginal smears. Further work is in progress to ascertain the possible significance of these findings.

CHRONIC ARTHRITIS IN LOWER MAMMALS. HERBERT FOX.

Skeletons of wild mammals collected either in their native habitats or from zoologic gardens show lesions which are comparable to those of chronic arthritis of man. In material which has been examined, these changes occurred chiefly among Carnivora and Artiodactyla. The infrahuman primates showed only

moderate disease, and none was found in Perissodactyla and Marsupialia. In Carnivora chronic arthritis was best developed in the cervical vertebrae, the anterior extremities and the posterior half of the spine. In Artiodactyla the disease was best developed in the thoracic spine and posterior extremities. Infrahuman primates were affected chiefly in the lower cervical, upper thoracic and lumbar regions of the spine and rarely in the appendicular skeleton. Positive correlation was lacking between disease of the teeth and arthritis in this material. The data suggest instead an association between the distribution of arthritic lesions and the anatomic and functional characters of the skeleton.

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Nov. 14, 1936

KORNEL TERPLAN, *President*

WILLIAM F. JACOBS, *Secretary*

SO-CALLED CONGENITAL ABSENCE OF THE LEFT CORONARY ARTERY IN A CHILD. S. SANES.

The type of anomaly which I wish to report is usually described in the literature under the title of congenital absence of the left or right coronary artery. References to this anomaly as an isolated cardiac defect are rather few. During the past hundred years there have appeared in the medical journals complete descriptions of only eleven cases; two of the descriptions are in the American literature.

On examination, the coronary artery which is reputedly absent cannot be found running from the aorta in the normal position; the normal orifice is completely lacking. In one group of cases the artery arises—either in itself or in its branches—along with or from the homologous coronary vessel. In a second group anatomic counterparts of the absent artery are not distinguishable as such, and the coronary artery which is considered to be present gives off branches and terminations that act as functional substitutes. Because of the anomalies in origin, the components of the coronary circulation assume variations also in caliber and course.

The patient was a white boy 3 years and 10 months of age. The final pathologic diagnosis comprised: acute diffuse glomerulonephritis; marked edema and congestion of both lungs with patchy areas of atelectasis of both lower lobes; slight bilateral hydrothorax; slight hydropericardium; slight ascites; edema of soft tissues; diffuse myocarditis; acute interstitial hepatitis; interstitial myositis (diaphragm); congestion of the brain; anomalous origin of the left coronary artery; anomalous courses of the left and right coronary arteries; synosteosis of the skull, and bilateral strabismus.

The left coronary artery was anomalous. No coronary orifice was seen in the left anterior sinus. In the right sinus of Valsalva was a large orifice which housed three moderate-sized openings. These were arranged so that two occupied a superior position, and one, a site midinferior to the other two. Two additional minute openings were present.

From the right superior opening emerged a vessel which corresponded to the right coronary artery. It gave off branches to the right side of the heart and the posterior wall of the left ventricle. The left superior opening provided the origin for a vessel that sank into the septum and reached the subepicardium at the interventricular sulcus, 2.2 cm. from the aortic ring. On the surface of the heart it conformed to the topography of the left anterior descending branch. From the midinferior opening a vessel passed behind the posterior and left aortic cusps to the left. It corresponded in part to the left circumflex branch. Histologically

there were recent diffuse myocarditis, parenchymatous and interstitial, capillary dilatation and recent hemorrhages.

The origin of the coronary arteries in the case described is not duplicated among the other eleven reported instances. This case is the first instance of so-called congenital absence of the left coronary artery in a child to be described. A histologic description of the myocardium has appeared in the records of but one case. The myocarditis in the present patient was interpreted on an infectious-toxic basis. The possibility of effects of the coronary anomaly on the myocardium was discussed.

SEROLOGIC DEMONSTRATION OF THE CAUSATIVE AGENT IN THE SPINAL FLUID IN CASES OF MENINGITIS. ERNEST WITEBSKY and ERWIN NETER.

In view of the significance and difficulties which at times may be encountered in the bacteriologic diagnosis of meningitis, attempts have been made toward employing serologic methods as an aid to the establishment of the diagnosis. Recently Rake and Scherp were able to demonstrate the presence of type-specific meningococcic substances in the spinal fluid in meningococcic meningitis. They added monovalent meningococcic antiserum to the supernatant fluid obtained by centrifugation of spinal fluid and observed type-specific precipitation. This method, however, has as yet not been used for practical diagnostic purposes, probably because of its lack of sensitivity.

The value of the complement fixation test in the diagnosis of meningococcic meningitis, as first employed by Russian investigators, was confirmed in a series of examinations carried out in our laboratory. In view of the possible therapeutic importance of the determination of the type of meningococcus involved, we applied the complement fixation test to the direct examination of the spinal fluid in meningococcic meningitis, employing monovalent type-specific meningococcic antiserum. Decreasing amounts of spinal fluid, without previous centrifugation, were mixed with monovalent type-specific meningococcic antiserums (rabbit). In two of three cases of meningococcic meningitis the diagnosis of the respective meningococci as to types could be easily established. In one case they were definitely of type 1; in the other, they were definitely of type 2. The polyvalent serum gave a marked reaction with the first spinal fluid. It failed, however, to react with the second one. It is important to note that the supernatant fluid obtained by centrifugation of the spinal fluid displayed a definitely weaker reaction than the whole spinal fluid.

The direct serologic examination of the spinal fluid in a case of influenzal meningitis revealed the causative agent. Two rabbit serums against Haemophilus influenzae and one rabbit serum against Haemophilus pertussis were at our disposal. Both the antiserums for influenza displayed a marked reaction with the spinal fluid (up to a dilution of 1:80) when examined by means of the complement fixation test, while the antiserum for H. pertussis did not react.

The experiments reported seem to encourage the employment of serologic methods as a means of establishing the diagnosis of meningitis in a larger series of examinations.

A CASE OF LUDWIG'S ANGINA DUE TO AN ESCULIN-FERMENTING HEMOLYTIC STREPTOCOCCUS (ENTEROCOCCUS). ERWIN NETER.

A girl 17 months of age was admitted to the Children's Hospital one day after an enlargement of the neck had been noted. Physical examination revealed a very large swelling in the right submaxillary region. The tonsils were only slightly enlarged, and no membranes or ulcerations could be seen in the throat. The temperature was 101 F. Otherwise the findings were negative. The blood showed 75 per cent hemoglobin, 17,050 leukocytes with 70 per cent segmented and 15 per cent nonsegmented polymorphonuclear cells, 12 per cent lymphocytes and 3 per cent monocytes. The next day the swelling of the neck increased remarkably and changed in character to a severe hemorrhagic-necrotic cellulitis involving the

whole neck and the anterior part of the upper region of the chest. The floor of the mouth was considerably raised. The temperature was 105 F. A culture for diphtheria bacilli was negative. Ludwig's angina was diagnosed. The child expired suddenly the same day.

Immediately after death, a culture of the lesion and of the heart's blood were made under sterile precautions. The fluid obtained from the hemorrhagic area contained a moderate amount of leukocytes and occasional gram-positive diplococci. The culture revealed a pure growth of a hemolytic streptococcus which was bile-insoluble and fermented esculin. This micro-organism, therefore, belonged to the group of Enterococcus and may be called Enterococcus haemolyticus. The blood culture showed the presence of more than 300 colonies of the esculin-fermenting hemolytic streptococcus.

Enterococci are normally present in the intestinal tract of man.

Undoubtedly such an esculin-fermenting streptococcus may also be pathogenic. There are only a few reports in the literature concerning the pathogenicity of hemolytic enterococci. In my case, Enterococcus haemolyticus caused Ludwig's angina.

Studies of the fibrinolytic properties of the exudate obtained from the lesion as well as of the isolated strain were also undertaken. According to previous examinations (Witebsky and Neter: *Proc. Soc. Exper. Biol. & Med.* **34**:858, 1936) there are two fibrinolysins produced by bacteria from human sources: one, originating from hemolytic streptococci, which dissolves human plasma clots but is ineffective toward animal plasma clots and which may be neutralized by specific antiserum, and one which inhibits coagulation of human as well as animal plasma and which is produced by a variety of micro-organisms, such as the enterococcus, Streptococcus viridans and Pneumococcus; this type of fibrinolysin (or, better, anti-coagulant) has not been neutralized by specific antiserums employed thus far. Fibrinolysin not only has been produced in vitro but has been encountered in vivo (Neter: *Proc. Soc. Exper. Biol. & Med.* **34**:735, 1936). In the case presented, the anticoagulant had the properties of that usually produced by enterococci and could be demonstrated in vitro as well as in vivo.

CONGENITAL BRONCHOGENIC CYST OF THE MEDIASTINUM IN AN INFANT. J. E. ALFORD.

I wish to present a case of congenital mediastinal cyst arising from the tracheobronchial tree in an infant.

Reports of mediastinal cystic tumors are not uncommon in the literature, but perhaps one of the rarest of such tumors is that which apparently originates in displaced germs of the tracheobronchial tree. Several cases have been described in the European journals, but only one has been found recorded in the American publications. The case which I report is of interest clinically because the cyst produced a clearcut clinical picture through which it played a primary part in the cause of death.

A girl 7 months and 2 weeks old was admitted to the hospital because of cough, dyspnea, cyanosis and breathing of an obstructive type, of four days' duration. She was said to have had bronchial asthma three months before. Physical examination revealed bronchopneumonia in the right and emphysema in the left side of the chest. An x-ray film confirmed this and in addition showed a deviation of the trachea to the right.

At autopsy the chief observations were diffuse confluent lobular and bronchial pneumonia of both lungs, alveolar and interstitial emphysema of the left upper lobe and a congenital mediastinal cyst with compression of the trachea and left major bronchus.

Description of Cyst.—The cyst was the size of a large cherry and filled out the upper angle between the trachea and the left major bronchus. It had thin walls, was translucent and contained a saliva-like mucoid material. Incorporated in the tissue about its wall was the left vagus nerve. The cyst caused inward

bulging of the trachea at its junction with the left major bronchus and almost completely occluded the opening of the latter.

Histologically, the wall contained cartilage, glands, connective tissue and smooth muscle fibers. The epithelium lining the lumen was columnar and in places had a definite brush border; there was much desquamation.

It is interesting to speculate as to the embryologic accident which caused the formation of this cyst. Probably during the cutting off of the respiratory premordium from the esophagus development was altered and the anlage of the cyst formed.

The attack of bronchial asthma was probably the first set of symptoms produced by the cyst and was undoubtedly due to partial occlusion of the bronchial orifice by the cyst.

The terminal illness was in all probability initiated by an acute infection of the upper respiratory tract which rapidly progressed to lobular pneumonia because the cyst was large enough to produce definite occlusion of the bronchus.

Of the ten cases described in the available literature, two directly resemble mine in that the cyst in question produced obstruction of one or both bronchi and the patient died of lobular pneumonia.

Books Received

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LES HYPERGLYCÉMIES. ÉTUDE CLINIQUE ET PHYSIOPATHOLOGIQUE. H. Warembourg, médecin des hôpitaux de Lille. Paper. Price, 65 francs. Pp. 584. Paris: Masson & Cie, 1936.

LA PRESSION MOYENNE DE L'HOMME. A L'ÉTAT NORMAL ET PATHOLOGIQUE. H. Vaquez and P. Gley. Paper. Price, 25 francs. Pp. 126, with 57 figures. Paris: Masson & Cie, 1936.

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LA PONCTION DE LA RATE. P. Emile Veil, médecin des hôpitaux; P. Isch-Wall, assistant à l'hôpital Tenon, and Suzanne Perles, chef de laboratoire à l'hôpital Tenon. Price, 35 francs. Pp. 148, with 23 figures. Paris: Masson & Cie, 1936.

A HUNDRED YEARS OF MEDICINE. Wyndham E. B. Lloyd, M.A. (Cantab.), M.R.C.S. (Eng.), D.P.H. (Eng.). Cloth. Price, 15 shillings. Pp. 344. London: Duckworth & Co., 1936.

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JUVENILE PARESIS. William C. Menninger, M.D., Meninger Clinic Monograph Series, no. 1. Cloth. Price, $3. Pp. 199, with 16 illustrations. Baltimore: Williams & Wilkins Company, 1936.

THE HARVEY LECTURES. Delivered Under the Auspices of the Harvey Society of New York, 1935-1936, Under the Patronage of the New York Academy of Medicine. Max Bergmann, M.D.; Robert M. Yerkes, M.D.; Peyton Rous, M.D.; B. A. Houssay, M.D.; John Farquhar Fulton, M.D.; Richard E. Shope, M.D.; Warren H. Lewis, M.D., and I. deBurgh Daly, M.D. Series XXXI. Cloth. Price, $4. Pp. 255, with illustrations. Baltimore: Williams & Wilkins Company, 1936.

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